	L #	Hits	Search Text	DBs	Time Stamp
1	L1	1607	chitinase$1	USPAT; US-PGPUB	2003/04/25 10:35
2	L2	13553	barley	USPAT; US-PGPUB	2003/04/25 10:35
3	L3	2061	glucanase$1	USPAT; US-PGPUB	2003/04/25 10:39
4	L4	145155	psi or protein adj synthesis adj inhibit$8	USPAT; US-PGPUB	2003/04/25 10:40
5	L5	1545	afp or antifungal adj protein	USPAT; US-PGPUB	2003/04/25 10:45
6	L6	85	1 near6 (serratia or marcesens)	USPAT; US-PGPUB	2003/04/25 10:45
7	L7	30	1 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
8	L8	101	3 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
9	L9	13	4 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
10	L10	25	5 near6 (aspergillus or giganteus)	USPAT; US-PGPUB	2003/04/25 10:47
11	L11	25	(6 and (7 or 8 or 9 or 10)) or (7 and (8 or 9 or 10)) or (8 and (9 or 10)) or (9 and 10)	USPAT; US-PGPUB	2003/04/25 11:46
12	L12	10	(6 or 7 or 8 or 9 or 10) same synerg$	USPAT; US-PGPUB	2003/04/25 11:13
13	L13	30	(6 or 7 or 8 or 9 or 10) same transgen$	USPAT; US-PGPUB	2003/04/25 14:19
14	L14	793	(1 same (3 or 4 or 5)) or (3 same (4 or 5)) or (4 same 5)	USPAT; US-PGPUB	2003/04/25 11:48
15	L15	39	14 same synerg$	USPAT; US-PGPUB	2003/04/25 11:49
16	L16	114	14 same transgen$	USPAT; US-PGPUB	2003/04/25 14:19

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	1607	chitinase$1	USPAT; US-PGPUB	2003/04/25 10:35
2	L2	13553	barley	USPAT; US-PGPUB	2003/04/25 10:35
3	L3	2061	glucanase$1	USPAT; US-PGPUB	2003/04/25 10:39
4	L4	145155	psi or protein adj synthesis adj inhibit$8	USPAT; US-PGPUB	2003/04/25 10:40
5	L5	1545	afp or antifungal adj protein	USPAT; US-PGPUB	2003/04/25 10:45
6	L6	85	1 near6 (serratia or marcesens)	USPAT; US-PGPUB	2003/04/25 10:45
7	L7	30	1 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
8	L8	101	3 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
9	L9	13	4 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
10	L10	25	5 near6 (aspergillus or giganteus)	USPAT; US-PGPUB	2003/04/25 10:47
11	L11	25	(6 and (7 or 8 or 9 or 10)) or (7 and (8 or 9 or 10)) or (8 and (9 or 10)) or (9 and 10)	USPAT; US-PGPUB	2003/04/25 11:08
12	L12	10	(6 or 7 or 8 or 9 or 10) same synerg$	USPAT; US-PGPUB	2003/04/25 11:09

PGPUB-DOCUMENT-NUMBER: 20020174452

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020174452 A1

TITLE: Monocot seeds with increased lignan content

PUBLICATION-DATE: November 21, 2002

US-CL-CURRENT: 800/284

APPL-NO: 09/ 944160

DATE FILED: August 30, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60230632 20000907 US

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit of priority from United States Provisional Patent Application No. 60/230,632, filed Sep. 7, 2000, under 35 U.S.C. .sctn. 119, which is incorporated herein by reference.

PGPUB-DOCUMENT-NUMBER: 20020168735

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168735 A1

TITLE: ANTIFUNGAL PROTEINS, DNA CODING THEREFORE, AND HOSTS INCORPORATING SAME

PUBLICATION-DATE: November 14, 2002

US-CL-CURRENT: 435/183, 435/320.1 , 435/325 , 435/69.1 , 514/12 , 530/387.1 , 536/23.2 , 800/295

APPL-NO: 09/ 258031

DATE FILED: February 25, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09258031 A1 19990225

parent continuation-of PCT/EP97/04923 19970904 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	96 202 466.7	1996EP-96 202 466.7	September 4, 1996
EP	97 200 831.2	1997EP-97 200 831.2	March 19, 1997

PGPUB-DOCUMENT-NUMBER: 20020088024

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020088024 A1

TITLE: Production of lysosomal enzymes in plants by transient expression

PUBLICATION-DATE: July 4, 2002

US-CL-CURRENT: 800/284, 435/200 , 435/235.1 , 435/320.1 , 435/410 , 536/23.2

APPL-NO: 09/ 993059

DATE FILED: November 13, 2001

RELATED-US-APPL-DATA:

child 09993059 A1 20011113

parent continuation-in-part-of 09626127 20000726 US PENDING

RELATED APPLICATIONS

[0001] This present application is a continuation-in-part of U.S. application Ser. No. 09/626,127, filed Jul. 26, 2000.

PGPUB-DOCUMENT-NUMBER: 20020039780

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020039780 A1

TITLE: Salicyclic acid inducible genes and promoters

PUBLICATION-DATE: April 4, 2002

US-CL-CURRENT: 435/219, 435/320.1 , 435/410 , 435/69.1 , 536/23.2

APPL-NO: 09/ 777207

DATE FILED: February 5, 2001

RELATED-US-APPL-DATA:

child 09777207 A1 20010205

parent continuation-of PCT/EP99/05581 19990802 US UNKNOWN

non-provisional-of-provisional 60095187 19980803 US

PGPUB-DOCUMENT-NUMBER: 20010020300

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010020300 A1

TITLE: TRANSGENIC PATHOGEN-RESISTANT ORGANISM

PUBLICATION-DATE: September 6, 2001

US-CL-CURRENT: 800/279

APPL-NO: 09/ 138873

DATE FILED: August 24, 1998

CONTINUED PROSECUTION APPLICATION: CPA

RELATED-US-APPL-DATA:

child 09138873 A1 19980824

parent division-of 08812025 19970306 US GRANTED

parent-patent 5804184 US

child 08812025 19970306 US

parent division-of 08457797 19950601 US GRANTED

parent-patent 5689045 US

child 08457797 19950601 US

parent continuation-of 08134416 19931008 US ABANDONED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	P 42 34 131.0	1992DE-P 42 34 131.0	October 9, 1992

US-PAT-NO: 6521435

DOCUMENT-IDENTIFIER: US 6521435 B1

TITLE: Nucleic acid sequences encoding cell wall-degrading enzymes and use to engineer resistance to Fusarium and other pathogens

DATE-ISSUED: February 18, 2003

US-CL-CURRENT: 435/206, 435/183 , 435/200 , 435/252.3 , 435/320.1 , 435/419 , 435/468 , 435/69.1 , 536/23.2 , 800/295 , 800/298 , 800/320.3

APPL-NO: 09/ 649747

DATE FILED: August 28, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications Nos. 60/224,946, filed Aug. 11, 2000 and 60/151,582, filed Aug. 30, 1999. The disclosure of each of said provisional application is incorporated herein by reference in its entirety.

US-PAT-NO: 6512166

DOCUMENT-IDENTIFIER: US 6512166 B1

TITLE: Combinations of fungal cell wall degrading enzyme and fungal cell membrane affecting compound

DATE-ISSUED: January 28, 2003

US-CL-CURRENT: 800/301, 514/12 , 800/279

APPL-NO: 08/ 611504

DATE FILED: March 5, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of application Ser. No. 08/499,164, filed Jul. 7, 1995, now abandoned, which is continuation of application Ser. No. 08/249,927, filed May 26, 1994, now U.S. Pat. No. 5,433,947, which is a continuation of application Ser. No. 07/990,609, filed Dec. 15, 1992, now U.S. Pat. No. 5,326,561. This application claims priority to Provisional Application Ser. No. 60/007567, filed Nov. 27, 1995. This is also a continuation-in-part of U.S. patent application Ser. No. 08/371,680, filed Dec. 21, 1994, issued as U.S. Pat. No. 6,020,540 which is a continuation-in-part of U.S. patent application Ser. No. 08/045,269, filed Apr. 14, 1993, now issued as U.S. Pat. No. 5,378,821, which is a continuation-in-part of U.S. patent application Ser. No. 07/919,784, filed Jul. 27, 1992, issued as U.S. Pat. No. 6,251,390, which is a continuation-in-part of U.S. patent application Ser. No. 07/716,134, filed Jun. 17, 1991, now issued as U.S. Pat. No. 5,173,419.

US-PAT-NO: 6465636

DOCUMENT-IDENTIFIER: US 6465636 B1
See image for Certificate of Correction

TITLE: Pathogen-inducible promoter

DATE-ISSUED: October 15, 2002

US-CL-CURRENT: 536/24.1, 435/320.1 , 435/410 , 800/278 , 800/279 , 800/295 , 800/301

APPL-NO: 09/ 647390

DATE FILED: September 29, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	98201024	April 1, 1998

PCT-DATA:
APPL-NO: PCT/EP99/02178
DATE-FILED: March 25, 1999
PUB-NO: WO99/50428
PUB-DATE: Oct 7, 1999
371-DATE: Sep 29, 2000
102(E)-DATE:Sep 29, 2000

US-PAT-NO: 6359196

DOCUMENT-IDENTIFIER: US 6359196 B1

TITLE: Germination-specific plant promoters

DATE-ISSUED: March 19, 2002

US-CL-CURRENT: 800/278, 435/320.1 , 435/419 , 435/6 , 536/23.1 , 536/24.1 , 536/24.3 , 800/288

APPL-NO: 09/ 404390

DATE FILED: September 23, 1999

US-PAT-NO: 6291647

DOCUMENT-IDENTIFIER: US 6291647 B1
See image for Certificate of Correction

TITLE: Antifungal proteins, DNA coding therefor, and hosts incorporating same

DATE-ISSUED: September 18, 2001

US-CL-CURRENT: 530/370, 435/418 , 435/419 , 530/300 , 530/350

APPL-NO: 08/ 687580

DATE FILED: November 20, 1996

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
NL	94200321	February 9, 1994

PCT-DATA:
APPL-NO: PCT/EP95/00488
DATE-FILED: February 9, 1995
PUB-NO: WO95/21929
PUB-DATE: Aug 17, 1995
371-DATE: Nov 20, 1996
102(E)-DATE:Nov 20, 1996

US-PAT-NO: 6284946

DOCUMENT-IDENTIFIER: US 6284946 B1

TITLE: Banana DNA associated with fruit development

DATE-ISSUED: September 4, 2001

US-CL-CURRENT: 800/278, 435/320.1 , 435/410 , 435/419 , 435/468 , 435/69.1 , 536/23.6 , 800/298

APPL-NO: 09/ 160351

DATE FILED: September 25, 1998

PARENT-CASE:

this application claims benefit to U.S. provisional application No. 60/060,062 filed Sep. 25, 1997.

US-PAT-NO: 6271438

DOCUMENT-IDENTIFIER: US 6271438 B1
See image for Certificate of Correction

TITLE: Transgenic pathogen-resistant plant

DATE-ISSUED: August 7, 2001

US-CL-CURRENT: 800/279, 424/94.2 , 424/94.61 , 435/200 , 435/209 , 435/320.1 , 435/69.1 , 514/12 , 536/23.2 , 800/301

APPL-NO: 09/ 138873

DATE FILED: August 24, 1998

PARENT-CASE:

This application is a divisional of prior application No. 08/812,025 filed Mar. 6, 1997, now U.S. Pat. No. 5,804,184, which, in turn, is a divisional of prior application No. 08/457,797, filed Jun. 1, 1995, now U.S. Pat. No. 5,689,045, which is a continuation of prior application No. 08/134,416, filed Oct. 6, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 34 131	October 9, 1992

US-PAT-NO: 6087560

DOCUMENT-IDENTIFIER: US 6087560 A

TITLE: Transgenic fungal resistant plants expressing chitinase and glucanase, process for obtaining, and recombinant polynucleotides for uses therein

DATE-ISSUED: July 11, 2000

US-CL-CURRENT: 800/301, 435/252.3 , 435/320.1 , 800/279 , 800/305 , 800/306 , 800/309 , 800/312 , 800/313 , 800/315 , 800/316 , 800/317 , 800/317.1 , 800/317.2 , 800/317.3 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3 , 800/321

APPL-NO: 08/ 801563

DATE FILED: February 18, 1997

PARENT-CASE:

This application is a continuation of U.S. Ser. No. 08/047,413 filed Apr. 19, 1993, U.S. Pat. No. 5,670,706, which is a continuation-in-part of U.S. Ser. No. 07/647,831 filed Jan. 29, 1991, abandoned, the disclosures of which are hereby incorporated herein by reference.

US-PAT-NO: 6066491

DOCUMENT-IDENTIFIER: US 6066491 A

TITLE: Process for obtaining fungal resistant plants with recombinant polynucleotides encoding .beta.-1,3-glucanase modified for apoplast targeting

DATE-ISSUED: May 23, 2000

US-CL-CURRENT: 435/252.3, 435/252.2 , 435/320.1

APPL-NO: 08/ 229050

DATE FILED: April 18, 1994

PARENT-CASE:

This application is a continuation of application Ser. No. 07/647,831, filed Jan. 29, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
NL	9000222	January 30, 1990

US-PAT-NO: 6020540

DOCUMENT-IDENTIFIER: US 6020540 A

TITLE: Gene encoding endochitinase

DATE-ISSUED: February 1, 2000

US-CL-CURRENT: 800/302, 435/209 , 435/252.3 , 435/320.1 , 435/418 , 435/419 , 536/23.1 , 536/23.2

APPL-NO: 08/ 371680

DATE FILED: December 21, 1994

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of U.S. Ser. No. 08/045,269, filed Apr. 14, 1993, now U.S. Pat. No. 5,378,821, which is a continuation-in-part of U.S. Ser. No. 07/919,784 filed Jul. 27, 1992, which is a continuation-in-part of U.S. Ser. No. 07/716,134, filed Jun. 17, 1991, now U.S. Pat. No. 5,173,419

And a continuation-in-part of U.S. Ser. No. 08/184,115, filed Jan. 21, 1994, now abandoned, which is a continuation-in-part of Ser. No. 08/049,390, filed Apr. 21, 1993, now U.S. Pat. No. 5,474,926, which is a continuation-in-part of U.S. Ser. No. 07/990,609, filed Dec. 15, 1992, now U.S. Pat. No. 5,326,561.

US-PAT-NO: 5994625

DOCUMENT-IDENTIFIER: US 5994625 A

TITLE: Antifungal chitin binding proteins and DNA coding therefor

DATE-ISSUED: November 30, 1999

US-CL-CURRENT: 800/279, 435/200 , 435/209 , 435/252.2 , 435/320.1 , 435/418 , 435/419 , 435/421 , 435/468 , 435/469 , 435/69.1 , 536/23.6 , 800/265 , 800/268 , 800/294 , 800/298 , 800/301

APPL-NO: 08/ 935886

DATE FILED: September 23, 1997

PARENT-CASE:

This application is a continuation of application(s) Ser. No. 08/411,640 filed on Apr. 5, 1995, now abandoned, which is International Application PCT/EP93/02790 filed on Oct. 5, 1993 and which designated the U.S.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	92203071	October 5, 1992
EP	93201370	May 13, 1993

US-PAT-NO: 5993808

DOCUMENT-IDENTIFIER: US 5993808 A

TITLE: Chitinase, DNA coding therefor and plants containing same

DATE-ISSUED: November 30, 1999

US-CL-CURRENT: 424/94.61, 435/200 , 435/209

APPL-NO: 08/ 591629

DATE FILED: February 15, 1996

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	93202425	August 17, 1993

PCT-DATA:
APPL-NO: PCT/EP94/02761
DATE-FILED: August 17, 1994
PUB-NO: WO95/05467
PUB-DATE: Feb 23, 1995
371-DATE: Feb 15, 1996
102(E)-DATE:Feb 15, 1996

US-PAT-NO: 5981844

DOCUMENT-IDENTIFIER: US 5981844 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: November 9, 1999

US-CL-CURRENT: 800/301, 435/320.1 , 435/419 , 800/279

APPL-NO: 08/ 994418

DATE FILED: December 19, 1997

PARENT-CASE:

This Application is a Continuation of U.S. application Ser. No. 08/456,430 filed Jun. 1, 1995, now U.S. Pat. No. 5,703,044, which is a division of Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a Continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a Continuation in-Part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned. Such applications are herein incorporated by reference.

US-PAT-NO: 5866788

DOCUMENT-IDENTIFIER: US 5866788 A

TITLE: Recombinant chitinase and use thereof as a biocide

DATE-ISSUED: February 2, 1999

US-CL-CURRENT: 800/302, 435/200 , 435/252.2 , 435/320.1 , 435/418 , 435/419 , 435/69.1 , 536/23.5

APPL-NO: 08/ 524051

DATE FILED: September 6, 1995

PARENT-CASE:

RELATED APPLICATION

This is a continuation-in-part of application Ser. No. 08/224,987, filed Apr. 8, 1994, now abandoned.

US-PAT-NO: 5804184

DOCUMENT-IDENTIFIER: US 5804184 A

TITLE: Transgenic pathogen-resistant organism

DATE-ISSUED: September 8, 1998

US-CL-CURRENT: 424/94.61, 424/94.2 , 435/200 , 435/209 , 514/12

APPL-NO: 08/ 812025

DATE FILED: March 6, 1997

PARENT-CASE:

This is a divisional of application No. 08/457,797, filed on Jun. 1, 1995, now U.S. Pat. No. 5,689,045, which is a continuation of Ser. No. 08/134,416, filed on Oct. 8, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 34 131.0	October 9, 1992

US-PAT-NO: 5703044

DOCUMENT-IDENTIFIER: US 5703044 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: December 30, 1997

US-CL-CURRENT: 514/12, 514/2 , 514/8 , 530/372 , 530/376

APPL-NO: 08/ 456430

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional of application Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-PAT-NO: 5689045

DOCUMENT-IDENTIFIER: US 5689045 A
See image for Certificate of Correction

TITLE: Transgenic pathogen-resistant plant

DATE-ISSUED: November 18, 1997

US-CL-CURRENT: 800/265, 435/200 , 435/209 , 435/320.1 , 435/69.1 , 435/70.1 , 47/DIG.1 , 536/23.2 , 536/23.6 , 536/23.7 , 800/279 , 800/301

APPL-NO: 08/ 457797

DATE FILED: June 1, 1995

PARENT-CASE:

This application is a continuation of application Ser. No. 08/134,416, filed on Oct. 8, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 34 131.0	October 9, 1992

US-PAT-NO: 5670706

DOCUMENT-IDENTIFIER: US 5670706 A

TITLE: Fungal resistant plants, process for obtaining fungal resistant plants and recombinant polynucleotides for use therein

DATE-ISSUED: September 23, 1997

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 800/294 , 800/301 , 800/317.4

APPL-NO: 08/ 047413

DATE FILED: April 19, 1993

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 07/647,831 filed 29 Jan. 1991, now abandoned.

US-PAT-NO: 5559034

DOCUMENT-IDENTIFIER: US 5559034 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: September 24, 1996

US-CL-CURRENT: 435/320.1, 435/252.3 , 435/69.1 , 514/12 , 514/2 , 514/8 , 530/372 , 530/376 , 536/22.1 , 536/23.1 , 536/23.6

APPL-NO: 08/ 457552

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional application of Ser. No. 08/178,708, filed Jan. 10, 1994, which is a continuation-in-part of Ser. No. 07,505,781, filed Apr. 6, 1990, now abandoned which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-PAT-NO: 5521153

DOCUMENT-IDENTIFIER: US 5521153 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: May 28, 1996

US-CL-CURRENT: 514/2, 514/12 , 514/8 , 530/372 , 530/376

APPL-NO: 08/ 178708

DATE FILED: January 10, 1994

PARENT-CASE:

This Application is a continuation-in-part application of U.S. application Ser. No. 07/505,781, filed Apr. 6, 1990, which is a continuation-in-part Application of U.S. application Ser. No. 07/104,755 filed Oct. 2, 1987, both now abandoned. Such applications are herein incorporated by reference .

US-PAT-NO: 6512166

DOCUMENT-IDENTIFIER: US 6512166 B1

TITLE: Combinations of fungal cell wall degrading enzyme and fungal cell membrane affecting compound

DATE-ISSUED: January 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harman; Gary E.	Geneva	NY	N/A	N/A
Lorito; Matteo	Salerno	N/A	N/A	IT
Di Pietro; Antonio	Cordoba	N/A	N/A	ES
Hayes; Christopher K.	Geneva	NY	N/A	N/A
Scala; Felice	Sorrento	N/A	N/A	IT
Kubicek; Christian P.	Vienna	N/A	N/A	AT

APPL-NO: 08/ 611504

DATE FILED: March 5, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of application Ser. No. 08/499,164, filed Jul. 7, 1995, now abandoned, which is continuation of application Ser. No. 08/249,927, filed May 26, 1994, now U.S. Pat. No. 5,433,947, which is a continuation of application Ser. No. 07/990,609, filed Dec. 15, 1992, now U.S. Pat. No. 5,326,561. This application claims priority to Provisional Application Ser. No. 60/007567, filed Nov. 27, 1995. This is also a continuation-in-part of U.S. patent application Ser. No. 08/371,680, filed Dec. 21, 1994, issued as U.S. Pat. No. 6,020,540 which is a continuation-in-part of U.S. patent application Ser. No. 08/045,269, filed Apr. 14, 1993, now issued as U.S. Pat. No. 5,378,821, which is a continuation-in-part of U.S. patent application Ser. No. 07/919,784, filed Jul. 27, 1992, issued as U.S. Pat. No. 6,251,390, which is a continuation-in-part of U.S. patent application Ser. No. 07/716,134, filed Jun. 17, 1991, now issued as U.S. Pat. No. 5,173,419.

US-CL-CURRENT: 800/301, 514/12 , 800/279

ABSTRACT:

A system for inhibiting the germination or growth of a fungus comprises (a) fungal cell wall degrading chitinolytic or glucanolytic enzyme and (b) antifungal cell membrane affecting: compound. Exemplified antifungal fungal cell membrane affecting compounds include flusilazole, miconazole, osmotin, gramicidin, valinomycin, phospholipase B, and trichorzianines. The system

components (a) and (b) may be supplemented with polyene macrolide antibiotic, antifungal epithiodiketopiperazine antibiotic (e.g., gliotoxin), fungal cell wall biosynthesis inhibitor (e.g., L-sorbose) and/or detergent. Embodiments include method of contacting a plant which expresses cell wall degrading enzyme with antifungal fungal cell membrane affecting compound.

17 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Other Reference Publication - OREF (46):

Brurberg et al., "Expression of a **Chitinase Gene From Serratia** marcescens In Lactococcus lactis and Lactobacillus plantarum," Appl Microbiol Biotechnol, 42:108-115 (1994).

Other Reference Publication - OREF (48):

Leah et al., "Identification of An Enhancer/Silencer Sequence Directing the Aleurone-Specific Expression of a **Barley Chitinase** Gene," The Plant Journal, 6:579-589 (1994).

Other Reference Publication - OREF (64):

Harpster et al., "Nucleoside Sequence of the **Chitinase B Gene of Serratia** marcescens," Nucleic Acids Research, 17:5395 (1989).

US-PAT-NO: 6087560

DOCUMENT-IDENTIFIER: US 6087560 A

TITLE: Transgenic fungal resistant plants expressing chitinase and glucanase, process for obtaining, and recombinant polynucleotides for uses therein

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cornelissen; Bernardus J. C.	Warmond		N/A	NL
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL
Meulenhoff; Elisabeth J. S.	Amsterdam		N/A	NL
van Roekel; Jeroen S. C.	Amsterdam		N/A	NL
Sela-Buurlage; Marianne	Amersfoort		N/A	NL
Beatrix	Leiden	N/A	N/A	NL
Vloemans; Alexandra Aleida	Lafayette	IN	N/A	N/A
Woloshuk; Charles Peter	Oegstgeest		N/A	NL
Bol; John Ferdinand	Leiden	N/A	N/A	NL
Linthorst; Hubertus J. M.				

APPL-NO: 08/ 801563

DATE FILED: February 18, 1997

PARENT-CASE:

This application is a continuation of U.S. Ser. No. 08/047,413 filed Apr. 19, 1993, U.S. Pat. No. 5,670,706, which is a continuation-in-part of U.S. Ser. No. 07/647,831 filed Jan. 29, 1991, abandoned, the disclosures of which are hereby incorporated herein by reference.

US-CL-CURRENT: 800/301, 435/252.3 , 435/320.1 , 800/279 , 800/305 , 800/306 , 800/309 , 800/312 , 800/313 , 800/315 , 800/316 , 800/317 , 800/317.1 , 800/317.2 , 800/317.3 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3 , 800/321

ABSTRACT:

Plants are provided with improved resistance against pathogenic fungi. They are genetically transformed with one or more polynucleotides which essentially comprise one or more genes encoding plant chitinases and .beta.-1,3-glucanases. Preferred are the intracellular forms of the said hydrolytic enzymes, especially preferred are those forms which are targeted to the apoplastic space of the plant by virtue of the modification of the genes encoding the said enzymes. Particularly preferred are plants exhibiting a relative overexpression of at least one gene encoding a chitinase and one gene encoding a .beta.-1,3-glucanase.

25 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Brief Summary Text - BSTX (33):

For example, genes encoding chitinases are known to be expressed in a developmentally regulated manner in, inter alia, tobacco flowers (Lotan et al., 1989). **Glucanases are known to occur in large quantities in seedlings of barley** (Swegle et al., 1989; Woodward & Fincher, 1982; Hoj et al., 1988, 1989).

Brief Summary Text - BSTX (41):

In U.S. Pat. No. 4,940,840, tobacco plants expressing a bacterial **chitinase gene (i.e. the chiA gene from Serratia** marcescens) have been shown to be less sensitive to the fungus Alternaria longipes.

Detailed Description Text - DETX (39):

Furthermore, chitinases and .beta.-1,3-glucanases can be isolated from pea, using chitosan as inducing compound (Mauch et al., 1984). Further analysis revealed the presence of at least five hydrolases, viz. two basic .beta.-1,3-glucanases and three basic chitinases (Mauch et al., 1988a). Intracellular and extracellular Chitinases which are serologically related to an intracellular chitinase from Sean can be isolated from Allium porrum L. (Spanu et al., 1989). Endochitinases and glucanases can also be isolated from maize, following inoculation of leaves with BMV (bromine mosaic virus) (Nasser et al., 1988). Chitinases which are serologically related to an intracellular endochitinase from bean (Swegle et al., 1989) can be isolated from barley (Hordeum vulgare). Also .beta.-1,3-**glucanases, as well as other classes of glucanases, can be isolated from barley** (Balance et al., 1976; Hoj et al., 1988, 1989). At least 4 different chitinases and 5 different .beta.-1,3-glucanases are known to exist in oat (Fink et al., 1988).

US-PAT-NO: 6066491

DOCUMENT-IDENTIFIER: US 6066491 A

TITLE: Process for obtaining fungal resistant plants with recombinant polynucleotides encoding .beta.-1,3-glucanase modified for apoplast targeting

DATE-ISSUED: May 23, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cornelissen; Bernardus Johannes Clemens	Warmond	N/A	N/A	NL
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL

APPL-NO: 08/ 229050

DATE FILED: April 18, 1994

PARENT-CASE:

This application is a continuation of application Ser. No. 07/647,831, filed Jan. 29, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
NL	9000222	January 30, 1990

US-CL-CURRENT: 435/252.3, 435/252.2 , 435/320.1

ABSTRACT:

Plants are provided with improved resistance against pathogenic fungi. They are genetically transformed with one or more polynucleotides which essentially comprise one or more genes encoding plant and .beta.-1,3-glucanases. Preferred are the intracellular forms of the said hydrolytic enzymes, especially preferred are those forms which are targeted to the apoplastic space of the plant by virtue of the modification of the genes encoding the said enzymes. Particularly preferred are plants exhibiting a relative overexpression of at least one gene encoding a .beta.-1,3-glucanase.

7 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Brief Summary Text - BSTX (31):

For example, genes encoding chitinases are known to be expressed in a developmentally regulated manner in, inter alia, tobacco flowers (Lotan et al., 1989). **Glucanases are known to occur in large quantities in seedlings of barley** (Swegle et al., 1989; Woodward & Fincher, 1982; Hoj et al., 1988, 1989).

Brief Summary Text - BSTX (39):

In U.S. Pat. No. 4,940,840, tobacco plants expressing a bacterial **chitinase gene (i.e. the chiA gene from Serratia** marcescens) have been shown to be less sensitive to the fungus Alternaria longipes.

Detailed Description Text - DETX (37):

Genes or cDNAs coding for the desired hydrolytic enzymes can for instance be isolated from tobacco (e.g. Legrand et al., 1987; Shinshi et al., 1987), tomato (Joosten et al., 1989), a basic intracellular chitinase can be isolated from potato (Gaynor, 1988; Kombrink et al., 1988), an extracellular chitinase can be isolated from cucumber (Metraux & Boller, 1986; Metraux et al., 1986), and both intracellular chitinases and glucanases can be isolated from bean (Broglie et al., 1986; Vogeli et al., 1988; Mauch & Staehelin, 1989). Furthermore, chitinases and .beta.-1,3-glucanases can be isolated from pea, using chitosan as inducing compound (Mauch et al., 1984). Further analysis revealed the presence of at least five hydrolases, viz. two basic .beta.-1,3-glucanases and three basic chitinases (Mauch et al., 1988a). Intracellular and extracellular Chitinases which are serologically related to an intracellular chitinase from bean can be isolated from Allium porrum L. (Spanu et al., 1989). Endochitinases and glucanases can also be isolated from maize, following inoculation of leaves with BMV (bromine mosaic virus) (Nasser et al., 1988). Chitinases which are serologically related to an intracellular endochitinase from bean (Swegle et al., 1989) can be isolated from barley (Hordeum vulgare). Also .beta.-1,3**-glucanases, as well as other classes of glucanases, can be isolated from barley** (Balance et al., 1976; Hoj et al., 1988, 1989). At least 4 different chitinases and 5 different .beta.-1,3-glucanases are known to exist in oat (Fink et al., 1988).

US-PAT-NO: 6020540

DOCUMENT-IDENTIFIER: US 6020540 A

TITLE: Gene encoding endochitinase

DATE-ISSUED: February 1, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harman; Gary E.	Geneva	NY	N/A	N/A
Tronsmo; Arne	Aas	N/A	N/A	NO
Hayes; Christopher K.	Geneva	NY	N/A	N/A
Lorito; Matteo	Salerno	N/A	N/A	IT
Klemsdahl; Sonja	.ANG.s	N/A	N/A	NO

APPL-NO: 08/ 371680

DATE FILED: December 21, 1994

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of U.S. Ser. No. 08/045,269, filed Apr. 14, 1993, now U.S. Pat. No. 5,378,821, which is a continuation-in-part of U.S. Ser. No. 07/919,784 filed Jul. 27, 1992, which is a continuation-in-part of U.S. Ser. No. 07/716,134, filed Jun. 17, 1991, now U.S. Pat. No. 5,173,419

And a continuation-in-part of U.S. Ser. No. 08/184,115, filed Jan. 21, 1994, now abandoned, which is a continuation-in-part of Ser. No. 08/049,390, filed Apr. 21, 1993, now U.S. Pat. No. 5,474,926, which is a continuation-in-part of U.S. Ser. No. 07/990,609, filed Dec. 15, 1992, now U.S. Pat. No. 5,326,561.

US-CL-CURRENT: 800/302, 435/209 , 435/252.3 , 435/320.1 , 435/418 , 435/419 , 536/23.1 , 536/23.2

ABSTRACT:

Two chitinases from Trichoderma harzianum P1 (ATCC 74058) show chitin-containing-fungus-inhibiting activity. One is an endochitinase and the other is a chitobiase. Both have molecular weights of 40 kDa and isoelectric points of 3.9. Endochitinases and chitobiases including the two purified from Trichoderma harzianum strain P1 demonstrate synergy with each other in antifungal effect. Isolated gene encoding for the endochitinase has the sequence set forth in the Sequence Listing as SEQ ID NO:1.

33 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Other Reference Publication - OREF (5):

Harpster et al., "Nucleotide Sequence of the **Chitinase B Gene of Serratia** marcescens," Nucleic Acids Research, 17:5395 (1989).

Other Reference Publication - OREF (126):

Brurberg et al., "Expression of a **Chitinase Gene From Serratia** marcescens In Lactococcus lactis and Lactobacillus plantarum," Appl Microbiol Biotechnol, 42:108-115 (1994).

Other Reference Publication - OREF (128):

Leah et al., "Identification of An Enhancer/Silencer Sequence Directing the Aleurone-Specific Expression of a **Barley Chitinase** Gene Gene," The Plant Journal, 6:579-589 (1994).

US-PAT-NO: 5994625

DOCUMENT-IDENTIFIER: US 5994625 A

TITLE: Antifungal chitin binding proteins and DNA coding therefor

DATE-ISSUED: November 30, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL
Sela-Buurlage; Marianne	Amersfoort	N/A	N/A	NL
Beatrix	Leiden	N/A	N/A	NL
Bres-Vloemans; Alexandra	Leiden	N/A	N/A	NL
Aleida	Haarlem	N/A	N/A	NL
Ponstein; Anne Silene	Warmond	N/A	N/A	NL
Apotheker-De Groot; Marion				
Cornelissen; Bernardus Johannes Clemens				

APPL-NO: 08/ 935886

DATE FILED: September 23, 1997

PARENT-CASE:

This application is a continuation of application(s) Ser. No. 08/411,640 filed on Apr. 5, 1995, now abandoned, which is International Application PCT/EP93/02790 filed on Oct. 5, 1993 and which designated the U.S.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	92203071	October 5, 1992
EP	93201370	May 13, 1993

US-CL-CURRENT: 800/279, 435/200 , 435/209 , 435/252.2 , 435/320.1 , 435/418 , 435/419 , 435/421 , 435/468 , 435/469 , 435/69.1 , 536/23.6 , 800/265 , 800/268 , 800/294 , 800/298 , 800/301

ABSTRACT:

Chimeric genes encoding antifungal chitin binding proteins (antifungal CBPs) with very low chitinase activity (10% or less than that of the class-I chitinases from tobacco). Also substantially pure DNA sequences encoding antifungal CBP are provided for the obtention of transgenic plants producing antifungal CBP. Plants expressing an antifungal CBP gene, optionally in combination with a plant expressible glucanase gene, show reduced susceptibility to fungi.

31 Claims, 5 Drawing figures

Exemplary Claim Number: 1,24

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Text - DETX (36):

From these observations we predict, that the antifungal CBPs according to the invention will show a synergistic effect with many other proteins that bind to chitin or degrade chitin such as chitinases. Examples of synergizing proteins that may be used in combination with antifungal CBPs according to the invention include, but are not limited to, .beta.-1,3-**glucanases and chitinases which are obtainable from barley** (Swegle M. et al., 1989, Plant Mol. Biol. 12, 403-412; Balance G. M. et al., 1976, Can. J. Plant Sci. 56, 459-466 Hoj P. B. et al., 1988, FEBS Lett. 230, 67-71; Hoj P. B. et al., 1989, Plant Mol. Biol. 13, 31-42 1989), bean (Boller T. et al, 1983, Planta 157, 22-31; Broglie K. E. et al. 1986, Proc. Natl. Acad. Sci. USA 83, 6820-6824; Vdgeli U. et al., 1988 Planta 174, 364-372); Mauch F. & Staehelin L. A., 1989, Plant-Cell 1, 447-457); cucumber (Metraux J. P. & Boller T. (1986), Physiol. Mol. Plant Pathol. 28, 161-169); leek (Spanu P. et al., 1989, Planta 177, 447-455); maize (Nasser W. et al., 1988, Plant Mol. Biol. 11, 529-538), oat (Fink W. et al., 1988, Plant Physiol. 88, 270-275), pea (Mauch F. et al. 1984, Plant Physiol. 76, 607-611; Mauch F. et al., 1988, Plant Physiol. 87, 325-333), poplar (Parsons, T. J. et al, 1989, P.N.A.S. 86, 7895-7899), potato (Gaynor J. J. 1988, Nucl. Acids Res. 16, 5210; Kombrink E. et al. 1988, Proc. Natl. Acad. Sci. USA 85, 782-786; Laflamme D. and Roxby R., 1989, Plant Mol. Biol. 13, 249-250), tobacco (e.g. Legrand M. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 6750-6754; Shinshi H. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 89-93), tomato (Joosten M. H. A. & De Wit P. J. G. M. 1989, Plant Physiol. 89, 945-951), wheat (Molano J. et al., 1979, J. Biol. Chem. 254, 4901-4907), and the like.

US-PAT-NO: 5981844

DOCUMENT-IDENTIFIER: US 5981844 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 994418

DATE FILED: December 19, 1997

PARENT-CASE:

This Application is a Continuation of U.S. application Ser. No. 08/456,430 filed Jun. 1, 1995, now U.S. Pat. No. 5,703,044, which is a division of Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a Continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a Continuation in-Part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned. Such applications are herein incorporated by reference.

US-CL-CURRENT: 800/301, 435/320.1 , 435/419 , 800/279

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

5 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and **barley AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn AFP preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they synergized with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, synergized with all AFP preparations, but synergy was particularly dramatic with corn-AFP preparations. Polyoxin synergized significantly with corn and wheat AFP preparations, while modest synergy was observed with combinations of amphotericin and AFP preparations from barley and corn. In contrast, no synergy was observed with papulocandin and AFP preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-AFP preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using synergy with nikkomycin to inhibit the growth of C albicans as an activity assay, the synergizing activity in corn-AFP preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the synergy assay. No synergy with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant synergy was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the synergizing enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The synergizing activity in these preparations may be due to minor components in the mixtures.

US-PAT-NO: 5703044

DOCUMENT-IDENTIFIER: US 5703044 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: December 30, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 456430

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional of application Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-CL-CURRENT: 514/12, 514/2 , 514/8 , 530/372 , 530/376

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

26 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and **barley AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn AFP preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they synergized with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, synergized with all AFP preparations, but synergy was particularly dramatic with corn-AFP preparations. Polyoxin synergized significantly with corn and wheat AFP preparations, while modest synergy was observed with combinations of amphotericin and AFP preparations from barley and corn. In contrast, no synergy was observed with papulocandin and AFP preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-AFP preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using synergy with nikkomycin to inhibit the growth of C. albicans as an activity assay, the synergizing activity in corn-AFP preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the synergy assay. No synergy with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant synergy was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the synergizing enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The synergizing activity in these preparations may be due to minor components in the mixtures.

US-PAT-NO: 5670706

DOCUMENT-IDENTIFIER: US 5670706 A

TITLE: Fungal resistant plants, process for obtaining fungal resistant plants and recombinant polynucleotides for use therein

DATE-ISSUED: September 23, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cornelissen; Bernardus J. C.	Warmond		N/A	NL
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL
Meulenhoff; Elisabeth J. S.	Amsterdam		N/A	NL
van Roekel; Jeroen S. C.	Amsterdam		N/A	NL
Sela-Buurlage; Marianne	Amersfoort		N/A	NL
Beatrix	Leiden	N/A	N/A	NL
Vloemans; Alexandra Aleida	Lafayette	IN	N/A	N/A
Woloshuk; Charles Peter	Oegstgeest		N/A	NL
Bol; John Ferdinand	Leiden	N/A	N/A	NL
Linthorst; Hubertus J. M.				

APPL-NO: 08/ 047413

DATE FILED: April 19, 1993

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 07/647,831 filed 29 Jan. 1991, now abandoned.

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 800/294 , 800/301 , 800/317.4

ABSTRACT:

Plants are provided with improved resistance against pathogenic fungi. They are genetically transformed with one or more polynucleotides which essentially comprise one or more genes encoding plant chitinases and .beta.-1,3-glucanases. Preferred are the intracellular forms of the said hydrolytic enzymes, especially preferred are those forms which are targeted to the apoplastic space of the plant by virtue of the modification of the genes encoding the said enzymes. Particularly preferred are plants exhibiting a relative overexpression of at least one gene encoding a chitinase and one gene encoding a .beta.-1,3-glucanase.

30 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Brief Summary Text - BSTX (32):

For example, genes encoding chitinases are known to be expressed in a developmentally regulated manner in, inter alia, tobacco flowers (Lotan et al., 1989). **Glucanases are known to occur in large quantities in seedlings of barley** (Swegle et al., 1989; Woodward & Fincher, 1982; Hoj et al., 1988, 1989).

Brief Summary Text - BSTX (40):

In U.S. Pat. No. 4,940,840, tobacco plants expressing a bacterial **chitinase gene (i.e. the chiA gene from Serratia** marcescens) have been shown to be less sensitive to the fungus Alternaria longipes.

Detailed Description Text - DETX (32):

Furthermore, chitinases and .beta.-1,3-glucanases can be isolated from pea, using chitosan as inducing compound (Mauch et al., 1984). Further analysis revealed the presence of at least five hydrolases, viz. two basic .beta.-1,3-glucanases and three basic chitinases (Mauch et al., 1988a). Intracellular and extracellular chitinases which are serologically related to an intracellular chitinase from bean can be isolated from Allium porrum L. (Spanu et al., 1989). Endochitinases and glucanases can also be isolated from maize, following inoculation of leaves with BMV (bromine mosaic virus) (Nasser et al., 1988). Chitinases which are serologically related to an intracellular endochitinase from bean (Swegle et al., 1989) can be isolated from barley (Hordeum vulgare). Also .beta.-1,3-**glucanases, as well as other classes of glucanases, can be isolated from barley** (Balance et al., 1976; Hoj et al., 1988, 1989). At least 4 different chitinases and 5 different .beta.-1,3-glucanases are known to exist in oat (Fink et al., 1988).

US-PAT-NO: 5559034

DOCUMENT-IDENTIFIER: US 5559034 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: September 24, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 457552

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional application of Ser. No. 08/178,708, filed Jan. 10, 1994, which is a continuation-in-part of Ser. No. 07,505,781, filed Apr. 6, 1990, now abandoned which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-CL-CURRENT: 435/320.1, 435/252.3 , 435/69.1 , 514/12 , 514/2 , 514/8 , 530/372 , 530/376 , 536/22.1 , 536/23.1 , 536/23.6

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

2 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and **barley AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn AFP preparations. Growth of the important human pathogen Candida albicanswas found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they synergized with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, synergized with all AFP preparations, but synergy was particularly dramatic with corn-AFP preparations. Polyoxin synergized significantly with corn and wheat AFP preparations, while modest synergy was observed with combinations of amphotericin and AFP preparations from barley and corn. In contrast, no synergy was observed with papulocandin and AFP preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-AFP preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using synergy with nikkomycin to inhibit the growth of C. albicans as an activity assay, the synergizing activity in corn-AFP preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the synergy assay. No synergy with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant synergy was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the synergizing enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The synergizing activity in these preparations may be due to minor components in the mixtures.

US-PAT-NO: 5521153

DOCUMENT-IDENTIFIER: US 5521153 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: May 28, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 178708

DATE FILED: January 10, 1994

PARENT-CASE:

This Application is a continuation-in-part application of U.S. application Ser. No. 07/505,781, filed Apr. 6, 1990, which is a continuation-in-part Application of U.S. application Ser. No. 07/104,755 filed Oct. 2, 1987, both now abandoned. Such applications are herein incorporated by reference .

US-CL-CURRENT: 514/2, 514/12 , 514/8 , 530/372 , 530/376

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

15 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and **barley AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn AFP preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they synergized with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, synergized with all AFP preparations, but synergy was particularly dramatic with corn-AFP preparations. Polyoxin synergized significantly with corn and wheat AFP preparations, while modest synergy was observed with combinations of amphotericin and AFP preparations from barley and corn. In contrast, no synergy was observed with papulocandin and AFP preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-AFP preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using synergy with nikkomycin to inhibit the growth of C. albicans as an activity assay, the synergizing activity in corn-AFP preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the synergy assay. No synergy with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant synergy was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the synergizing enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The synergizing activity in these preparations may be due to minor components in the mixtures.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	1607	chitinase$1	USPAT; US-PGPUB	2003/04/25 10:35
2	L2	13553	barley	USPAT; US-PGPUB	2003/04/25 10:35
3	L3	2061	glucanase$1	USPAT; US-PGPUB	2003/04/25 10:39
4	L4	145155	psi or protein adj synthesis adj inhibit$8	USPAT; US-PGPUB	2003/04/25 10:40
5	L5	1545	afp or antifungal adj protein	USPAT; US-PGPUB	2003/04/25 10:45
6	L6	85	1 near6 (serratia or marcesens)	USPAT; US-PGPUB	2003/04/25 10:45
7	L7	30	1 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
8	L8	101	3 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
9	L9	13	4 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
10	L10	25	5 near6 (aspergillus or giganteus)	USPAT; US-PGPUB	2003/04/25 10:47
11	L11	25	(6 and (7 or 8 or 9 or 10)) or (7 and (8 or 9 or 10)) or (8 and (9 or 10)) or (9 and 10)	USPAT; US-PGPUB	2003/04/25 11:08
12	L12	10	(6 or 7 or 8 or 9 or 10) same synerg$	USPAT; US-PGPUB	2003/04/25 11:09

PGPUB-DOCUMENT-NUMBER: 20020065408

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020065408 A1

TITLE: HMG2 promoter expression system and post-harvest production of gene products in plants and plant cell cultures

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cramer, Carole Lyn	Blacksburg	VA	US	
Weissenborn, Deborah Louise	Blacksburg	VA	US	

APPL-NO: 09/ 902653

DATE FILED: July 12, 2001

RELATED-US-APPL-DATA:

child 09902653 A1 20010712

parent continuation-of 08890624 19970709 US ABANDONED

child 08890624 19970709 US

parent continuation-of 08282581 19940729 US GRANTED

parent-patent 5670349 US

child 08282581 19940729 US

parent continuation-in-part-of 08100816 19930802 US ABANDONED

US-CL-CURRENT: 536/24.1, 536/23.6 , 800/278

ABSTRACT:

The invention relates in part to plant HMG2 HMGR genes and in part to the "post-harvest" production method of producing gene product of interest in plant tissues and cultures. The HMG2 promoter elements are responsive to pathogen-infection, pest-infestation, wounding, or elicitor or chemical treatments. The HMG2 elements are also active in specialized tissues of the plant including pollen and mature fruits. HMG2 promoter elements and HMG2-derived promoters can be advantageously used to drive the expression of disease and pest resistance genes, whereby transgenic plants containing such gene constructs would be resistant to the targeted disease and pest. In particular, the HMG2 gene expression system can be utilized in developing

nematode resistant plants. The post-harvest production method of the invention utilizes plant tissues and cell cultures of plants or plant cells engineered with a expression construct comprising an inducible promoter, such as the HMG2 promoter, operably linked to a gene of interest. Production of the desired gene product is obtained by harvesting, followed by inducing and processing the harvested tissue or culture. The post-harvest production method may be advantageously used to produce direct or indirect gene products that are labile, volatile, toxic, hazardous, etc.

[0001] This application is a continuation-in-part of co-pending application Ser. No. 08/100,816 filed Aug. 2, 1993, which is hereby incorporated by reference in its entirety.

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Detail Description Table CWU - DETL (1):

3TABLE 3 Inducible Plant Genes with Potential for Post-harvest Induction and Accumulation of Transgene Products. An asterisk designates those genes for which promoters have been isolated and characterized. References represent one to two representative references or relevant review article and are not intended to be exhaustive. Genes/gene products Functions Sources of clones Defense-Response Genes.sup.a1 Phytoalexin biosynthesis Phenylpropanoid phytoalexin *Phenylalanine ammonia lyase (PAL).sup.2 Enzyme, central pathway Bean, parsley, potato, tomato 4-Coumarate CoA ligase (4CL).sup.3 Enzyme, central pathway Parsley, potato *Chalcone synthase (CHS).sup.4,5 Enzyme, Isoflavanoid branch Bean, soybean, parsley Chalcone isomerase (CHI).sup.6 Enzyme, Isoflavanoid branch Bean Resveratrol (stilbene) synthase.sup.7 Enzyme, Isoflavanoid branch Grapevine, peanut Isoflavone reductase (IFR).sup.8 Enzyme, Isoflavanoid branch Alfalfa Terpenoid phytoalexins *HMG-CoA reductase (HMG).sup.9,10 Enzymes, central pathway Tomato, tobacco, potato, rice Casbene synthetase.sup.11 Casbene biosynthesis Castor bean Cell wall components Lignin *Phenylalanine ammonia lyase See above Cinnamyl alcohol dehydrogenase (CAD).sup.12 Lignin biosyn. Tobacco Caffeic acid o-methyltransferase.sup.13 Lignin biosyn. Alfalfa, tobacco Lignin-forming peroxidase.sup.14 Lignin polymerization Tobacco, wheat Hydroxyproline-rich glycoproteins (HRGP).sup.15,16 Structural protein Bean, tomato Glycine-rich proteins (GRP).sup.15 Structural protein Bean, potato, pea, rice Thionins.sup.17 Antifungal Barley Hydrolases, lytic enzymes *Chitinases (PR-P, PR-Q).sup.18-20 Class I chitinase, basic Vacuolar, antifungal Tobacco, bean, tomato Class I and II chitinase, acidic Extracellular, antifungal Bean Class II chitinase Bifunctional lysozyme, Cucumber, tobacco, **chitinase barley,** petunia *.beta.-1,3-Glucanase.sup.21 Antifungal, chitinase Bean, tobacco, potato, **synergist** pea, rice, Arabidopsis .beta.-fructosidase.sup.22 Antifungal invertase Tomato Others *Proteinase inhibitors (PI-I, PI-II).sup.23,24 Trypsin-, chymotrypsin- Potato, tomato inhibitors Superoxide dismutase (SOD).sup.25 Anti-oxidant enzyme Tobacco, maize, tomato Lipoxygenase.sup.26 Lipid peroxidation, Arabidopsis jasmonate biosyn. Additional "pathogenesis-related" prot. *PR1 family, PR2, PR3.sup.27-29 Unknown Tobacco, bean, parsley, pea Osmotin, PR5.sup.30-32 Antifungal, thaumatin-like Tobacco, maize Ubiquitin.sup.33 Protein degradation

Potato Wound-Inducible Genes.sup.a *win1, *win2 (hevein-like).sup.34 Chitin-binding prot. Potato (hevein, rubber tree) wun1, wun2.sup.35 Unknown Potato *nos, nopaline synthase.sup.36 Agrobacterium nutr. Agrobacterium tumefaciens ACC synthase.sup.37 Ethylene biosynthesis Tomato, squash HMG-CoA reductase hmg1.sup.38 Sterol/alkaloid synth. Potato 3-deoxy--D-arabino-heptulosonate- Lignin biosyn. Potato, tomato 7-phosphate synthase.sup.39 HSP70.sup.33 Heat-shock protein, Potato chaparone Salicylic acid inducible.sup.40 acid peroxidase.sup.14 Lignin-forming Tobacco PR-proteins.sup.40,41 (see above) Tobacco Glycine-rich protein.sup.41 Cell wall protein Tobacco Methyl jasmonate inducible *vspB.sup.42 Vacuolar storage prot. Soybean Proteinase inhibitors I and II.sup.43 Trypsin, chymotrypsin inhib. Potato, tomato Heat-shock genes.sup.43 HSP70.sup.33 Chaperonin Potato Ubiquitin (see above) Cold-stress inducible.sup.44 Drought, salt stress.sup.45 Osmotin.sup.30-32 Desiccation tolerance Tobacco, maize Hormone inducible Gibberellin .alpha.-amylase.sup.46 Starch degradation Barley Abscisic acid.sup.45,47 EM-1, RAB, LEA genes.sup.45 Unknown, embryogenesis Wheat, rice, maize, cotton Ethylene Chitinase, phytoalexin biosyn. genes (see above) .sup.aGenes are transcriptionally activated in response to pathogens, defense elicitors, wounding and in some cases methyl jasmonate, salicylic acid, HgCl.sub.2 or H.sub.2O.sub.2.

References: .sup.1Cramer et al., 1993, J. Nematol. 25:507-518. .sup.2Lois et al., 1989, EMBO J. 8:1641-1648. .sup.3Becker-Andre et al., 1991, J. Biol. Chem. 266:8551-8559. .sup.4Arias et al., 1993, Plant Cell 5:485-496. .sup.5Doerner et al., 1990, Bio/Technology 8:845-848. .sup.6Mehdy et al., 1987, EMBO J. 6:1527-1533. .sup.7Hain et al., 1993, Nature 361:153-156. .sup.8Paiva et al., 1991, Plant Mol. Biol. 17:653-667. .sup.9Park et al, 1992, Plant Mol. Biol. 20:327-331. .sup.10Yang et al., 1991, Plant Cell 3:397-405. .sup.11Lois et al., 1990, Arch. Biochem. Biophys. 276:270-277. .sup.12Schuch et al., 1991, 3rd Int. Cong. Plant Mol. Biol., Tucson, Az., Abstract 1653. .sup.13Jaeck et al., 1992, Mol. Plant-Microbe Interact. 5:294-300. .sup.14Lagrimini et al., 1987, Proc. Natl. Acad. Sci. USA 84:7542-7546. .sup.15Showalter et al., 1992, Plant Mol. Biol. 19:205-215. .sup.16Wycoff et al., 1992, in D. P. S. Verma, ed., Molecular Signals in Plant-Microbe Communication, Boca Raton, FL:CRC Press. pgs. 407-422. .sup.17Bohlmann et al., 1988, EMBO J. 7:1559-1565. .sup.18Hedrick et al., 1988, Plant Physiol. 86:182-186. .sup.19Roby et al., 1990, Plant Cell 2:999-1007. .sup.20Samac and Shah, 1991, Plant Cell 3:1063-1072. .sup.21Legrand et al., 1987, Proc. Natl. Acad. Sci. USA 84:6750-6754. .sup.22Benhamou et al., 1991, Plant Physiol. 97:739-750. .sup.23Ryan, C. A., 1990, Annu. Rev. Phytopathol. 28:425-449. .sup.24Keil et al., 1989, EMBO J. 8:1323-1330. .sup.25Bowler et al., 1989, EMBO J. 8:31-38. .sup.26Melan et al., 1993, Plant Physiol. 101:441-450. .sup.27Corelissen et al., 1986, EMBO J. 5:37-40. .sup.28Meler et al., 1991, Plant Cell 3:309-315. .sup.29Sharma et al., 1992, Mol. Plant-Microbe Interact. 5:89-95. .sup.30Cornelissen et al., 1986, Nature 321:531-532. .sup.31Stinizi et al., 1991, Physiol. Mol. Plant Pathol. 38:137-146. .sup.32Kononowicz et al., 1992, Plant Cell 4:513-524. .sup.33Rickey and Belknap, 1991, Plant Mol. Biol. 16:1009-1018. .sup.34Weiss and Bevan, 1991, Plant Physiol. 96:943-951. .sup.35Logemann et al., 1988, Proc. Natl. Acad. Sci. USA 85:1136-1140. .sup.36An et al., 1990, Plant Cell 2:225-233. .sup.37Li et al., 1992, Plant Mol. Biol. 18:477-487. .sup.38Chol et al., 1992, Plant Cell 4:1333-1344. .sup.39Dyer et al., 1989, Proc. Natl. Acad. Sci. USA 86:7370-7373. .sup.40Ward et al., 1991, Plant Cell 3:1085-1094. .sup.41Van de Rhee et al., 1990, Plant Cell 2:357-366. .sup.42Mason et al., 1993, Plant

Cell 5:241-251. .sup.43Ho and Sachs, 1989, In: Stump_and Conn, eds., The Biochemistry of Plants: A Comprehensive Treatis, Vol. 15, pgs. 347-377. .sup.44Thomashow, 1992, In: Scandalios and Wright, eds., Genomic Responses to Environmental Stress, Adv. in Genetics, Vol. 28, pgs. 99-131. .sup.45Skriver and Mundy, 1990, Plant Cell 2:503-512. .sup.46Gubler and Jacobsen, 1992, Plant Cell 4:1435-1441. .sup.47Chandler and Robertson, 1994, Annu. Rev. Plant Physiol. Plant Mol. Biol. 45:113-142.

US-PAT-NO: 6521435

DOCUMENT-IDENTIFIER: US 6521435 B1

TITLE: Nucleic acid sequences encoding cell wall-degrading enzymes and use to engineer resistance to Fusarium and other pathogens

DATE-ISSUED: February 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Okubara; Patricia A.	Richmond	CA	N/A	N/A
Blechl; Ann E.	Albany	CA	N/A	N/A
Hohn; Thomas M.	Chapel Hill	NC	N/A	N/A
Berka; Randy M.	Davis	CA	N/A	N/A

APPL-NO: 09/ 649747

DATE FILED: August 28, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications Nos. 60/224,946, filed Aug. 11, 2000 and 60/151,582, filed Aug. 30, 1999. The disclosure of each of said provisional application is incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/206, 435/183 , 435/200 , 435/252.3 , 435/320.1 , 435/419 , 435/468 , 435/69.1 , 536/23.2 , 800/295 , 800/298 , 800/320.3

ABSTRACT:

The invention is directed to nucleic acid sequences derived from Fusarium fungal genes which encode the cell wall-degrading enzymes glucanase, endochitinase, and exochitinase; isolated polypeptides having glucanase, endochitinase or exochitinase activity; recombinant nucleic acid molecules, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides, including expression in plant cells to confer or enhance a plant's resistance to Fusarium and other pathogens.

11 Claims, 20 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 20

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Brief Summary Text - BSTX (18):

Some of the chitinases and .beta.1,3-glucanases produced by naturally occurring bacteria and fungi have anti-Fusarium properties (Mitchell and Alexander 1961; Michael and Nelson 1972, Cherif and Benhamou 1990). Glucanases and chitinases from plants can degrade isolated cell walls of Fusarium solani (Mauch et al. 1988). Chitinases from tobacco were inhibitory to the growth of F. oxysporum (Yun et al. 1996) and F. solani (Sela-Buurlage et al. 1993) in culture. Krishnaveni et al. (1999b) have described three chitinases from sorghum seeds that inhibit the growth of F. moniliforme. The **synergistic** action of chitinases and glucanases against fungal pathogens is widely reported (reviewed in Graham and Sticklen 1994, Van Loon 1997). For instance, Mauch et al. (1988) observed that a chitinase and a .beta.1,3-glucanase from pea wee active against a wide range of fungi. Melchers et al. (1994) reported the combined action of a Class V endochitinase plus a Class I .beta.1,3-glucanase, both from tobacco, **synergistically** inhibited the growth of F. solani. Expression of a tobacco acidic chitinase with a tobacco .beta.1,3-glucanase conferred resistance to F. oxysporum in tomato, whereas each protein had much less effect when expressed singly (Jongedijk et al. 1995). Likewise, the fungal pathogen Cercospora nicotiana was curtailed on tobacco expressing both a rice basic chitinase and an alfalfa acidic .beta.-1,3-glucanase (Zhu et al. 1994). The **synergistic** action of a **barley Class II chitinase and a barley Class II b-1,3-glucanase** conferred protection to tobacco against Rhizoctonia solani (Jach et al. 1995). This **chitinase in combination with a barley** ribosome inactivating protein also inhibited R. solani infection.

Detailed Description Text - DETX (347):

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Zoina, S. Tuzun and F. Scala (1998). Genes from the mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. Proc. Natl. Acad. Sci. 95: 7860-7865 Luehrsen, K. R. and V. Walbot (1994). The impact of AUG start codon context on maize gene expression in vivo. Plant Cell Rep. 13: 454-458 Malehorn, D. E., K. J. Scott and D. M. Shah (1993). Structure and expression of a **barley acidic .beta.-glucanase** gene. Plant Mol. Biol. 22: 347-360 Mauch F., B. Mauch-Mani and T. Boller (1988). Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and .beta.-1,3-glucanase. Plant Physiol. 88: 936-942 McCreath, K. J. and G. W. Gooday (1992). A rapid and sensitive microassay for determination of chitinolytic activity. J Microbiol Meth 14: 229-237 McCue, K. F. and A. D. Hanson (1990). Drought and salt tolerance: towards understanding and application. Trends Biotech. 8: 358-362 McKay, R. and J. B. Loughnane (1945), Observations on Gibberella saubinetti (Mont.) Sacc. on cereals in Ireland in 1943 and 1944. Sci. Proc.R. Dublin Soc. 24: 9-18 Melchers, L. S., M. Apotheker-de Groot, J. A. van der Knaap, A. S. Ponstein, M. B. Sela-Buurlage, J. F. Bol, B. J. C. Cornelissen, P. J. M. van den Elzen, and H. J. M. Linthorst (1994). A new class of tobacco chitinases homologous to bacterial exo-chitinases displays antifungal activity. Plant J. 5: 469-480 Michael, A. H. and Nelson, P. E. (1972). Antagonistic effect of soil bacteria on Fusarium roseum culmorum. Phytopathology 62: 1052-1056 Mitchell, R. and Alexander, M. (1961). The mucolytic phenomenon and biological control of Fusarium in soil. Nature 190: 109-110 Molina, A., A. Segura and F. Garcia-Olmedo (1993). Lipid transfer proteins (nsLTPs) from barley and maize leaves are potent inhibitors of bacterial and fungal plant pathogens. FEBS Lett. 316: 119-122 Nehra, N. S., R. N. Chibbar, N. Leung, K. Caswell, C. Mallard, L. Steinhauer, M. Baga and K. K. Kartha (1994). Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs. Plant J. 5: 285-297 Parry, D. W., P. Jenkinson and L. McLeod (1995). Fusarium ear blight (scab) in small grain cereals--a review. Plant Pathology 44: 207-238 Payne, G., E. Ward, T. Gaffney, P. A. Goy, M. Moyer, A. Harper. F. Meins, Jr. and J. Ryals (1990). Evidence for a third class of .beta.-1,3-glucanase in tobacco. Plant Mol. Biol. 15: 797-808 Pearce, R. B., R. N. Strange and H. Smith (1976). Glycinebetaine and choline in wheat: distribution and relation to infection by Fusarium graminearum. Phytochemistry 15: 953-954 Pearson, W. R. (1990). Rapid and sensitive sequence comparison with FASTP and FASTA. Methods Enzymology 183:63-98. Peterbauer, C. K., M. Lorito, C. K. Hayes, G. E. Harman and C. P. Kubicek (1996). Molecular cloning and expression of the nag1 gene (N-acetyl-.beta.-D-glucosaminidase-encoding gene) from Trichoderma harzianum P1. Curr. Genet. 30: 325-331 Pugh, G. W., H. Johann and J. G. Dickson (1933). Factors affecting infection of wheat heads by Gibberella saubinetii. J. Agr. Res. 46: 771-797 Sambrook, J., E. F. Fritsch and T. Maniatis (1989). Molecular Cloning, A Laboratory Manual, vol. 1-3 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Sanger, F., S. Nicklen and A. R. Coulsen (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad, Sci. 74: 5463-5467 Seetharaman, K., E. Whitehead, N. P. Keller, R. D. Wanishka, and L. W. Rooney ((1997). In vitro activity of sorgum seed antifungal proteins against grain mold pathogens. J. Agric. Food Chem. 45: 3666-3671 Sela-Buurlage, M. B., A. S. Ponstein, S. A. Bres-Vloemans, L. S. Melchers, P. J. M. van der Elzen and B. J. C. Comelissen (1993). Only specific tobacco (Nicotiana tabacum) chitinases and .beta.-1,3-glucanases exhibit antifungal activity. Plant Physiol. 101: 857-863 Simmons, C. R. (1994). The

physiology and molecular biology of plant 1,3-.beta.-D-glucanases and 1,3;1,4-.beta.-D-glucanases. Crit. Rev. Plant Sci. 13: 325-387 Sivan, A. and I. Chet (1989a). Cell wall composition of Fusarium oxysporum. Soil Biol. Biochem. 21: 869-871 Sivan A and I. Chet (1989b). Degradation of fungal cell walls by lytic enzymes of Trichoderma harzianum. J. Gen. Microbiol. 135: 675-682 Spratt, B. G., P. J. Hedge, S. te Heesen, A. Edelman and J. K. Broome-Smith (1986). Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8 and pEMBL9. Gene 41: 337-342 Srivastava, A. K., G. Defago, T. Boller (1985). Secretion of chitinase by Aphanocladium album, a hyperparasite of wheat rust. Experientia 41: 1612-1613 Strange, R. N., J. R. Majer and H. Smith (1974). The isolation and identification of choline and betaine as the two major components in anthers and wheat germ that stimulate Fusarium graminearum in vitro. Physiol. Plant Path. 4: 277-290 Strange, R. N. and H. Smith (1971). A fungal growth stimulant in anthers which predisposes wheat to attack by Fusarium graminearum. Physiol. Plant Path.

US-PAT-NO: 6137030

DOCUMENT-IDENTIFIER: US 6137030 A
See image for Certificate of Correction

TITLE: Pap mutants that exhibit anti-viral and/or anti-fungal activity in plants

DATE-ISSUED: October 24, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tumer; Nilgun E.	Belle Mead	NJ	N/A	N/A

APPL-NO: 09/ 005273

DATE FILED: January 9, 1998

PARENT-CASE:

This Application is a Continuation of PCT/US96/11546 filed Jul. 11, 1996, which is a Continuation-In-Part of U.S. application Ser. No. 08/500,611 filed Jul. 11, 1995, now U.S. Pat. No. 5,756,322, and application Ser. No. 08/500,694 filed Jul. 11, 1995, now U.S. Pat. No. 5,880,329.

US-CL-CURRENT: 800/279

ABSTRACT:

Disclosed are PAP mutants having reduced phytotoxicity compared to wild-type PAP, and which confer broad spectrum resistance to viruses and/or fungi in plants. One group of PAP mutants is characterized by at least one amino acid substitution in the N-terminus of mature PAP, such as the Glycine 75 residue or the Glutamic acid 97 residue; two groups of additional PAP mutants are characterized by truncations in the N-terminal region of mature PAP and truncations or amino acid substitutions in the C-terminal region of mature PAP, respectively; and a further group are enzymatically inactive which still exhibit anti-fungal properties. Also disclosed are DNA molecules encoding the PAP mutants, mutant PAP DNA constructs, and transgenic seed and plants containing the DNAs. Further disclosed are methods for identifying PAP mutants having reduced phytotoxicity, as well as isolated and purified PAP mutants identified by the method.

26 Claims, 0 Drawing figures

Exemplary Claim Number: 1,24

---------- KWIC ---------

Brief Summary Text - BSTX (8):

In addition to the studies on virus resistance in plants, RIPs have been studied in conjunction with fungal resistance. For example, Logeman et al., Bio/Technology 10:305-308 (1992), report that a RIP isolated from barley endosperm provided protection against fungal infection to transgenic tobacco plants. The combination of **barley endosperm RIP and barley class-II chitinase has provided synergistic** enhancement of resistance to Rhizoctonia solani in tobacco, both in v and in vivo. See, e.g., Lea et al., supra; Mauch et al., supra; Zhu et al., supra; and Jach et al., The Plant Journal 8:97-109 (1995). PAP, however, has not shown antifungal activity in vitro. See Chen et al., Plant Pathol. 40:612-620 (1991), which reports that PAP has no effect on the growth of the fungi Phytophthora infestans, Colletotrichum coccodes, fusarium solani, fusarium sulphureum, Phoma foreata and Rhizoctonia solani, in vitro.

US-PAT-NO: 5994625

DOCUMENT-IDENTIFIER: US 5994625 A

TITLE: Antifungal chitin binding proteins and DNA coding therefor

DATE-ISSUED: November 30, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL
Sela-Buurlage; Marianne Beatrix	Amersfoort	N/A	N/A	NL
	Leiden	N/A	N/A	NL
Bres-Vloemans; Alexandra Aleida	Leiden	N/A	N/A	NL
	Haarlem	N/A	N/A	NL
Ponstein; Anne Silene	Warmond	N/A	N/A	NL
Apotheker-De Groot; Marion				
Cornelissen; Bernardus Johannes Clemens				

APPL-NO: 08/ 935886

DATE FILED: September 23, 1997

PARENT-CASE:

This application is a continuation of application(s) Ser. No. 08/411,640 filed on Apr. 5, 1995, now abandoned, which is International Application PCT/EP93/02790 filed on Oct. 5, 1993 and which designated the U.S.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	92203071	October 5, 1992
EP	93201370	May 13, 1993

US-CL-CURRENT: 800/279, 435/200 , 435/209 , 435/252.2 , 435/320.1 , 435/418 , 435/419 , 435/421 , 435/468 , 435/469 , 435/69.1 , 536/23.6 , 800/265 , 800/268 , 800/294 , 800/298 , 800/301

ABSTRACT:

Chimeric genes encoding antifungal chitin binding proteins (antifungal CBPs) with very low chitinase activity (10% or less than that of the class-I chitinases from tobacco). Also substantially pure DNA sequences encoding antifungal CBP are provided for the obtention of transgenic plants producing antifungal CBP. Plants expressing an antifungal CBP gene, optionally in combination with a plant expressible glucanase gene, show reduced susceptibility to fungi.

31 Claims, 5 Drawing figures

Exemplary Claim Number: 1,24

Number of Drawing Sheets: 5

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Detailed Description Text - DETX (36):

From these observations we predict, that the antifungal CBPs according to the invention will show a **synergistic** effect with many other proteins that bind to chitin or degrade chitin such as chitinases. Examples of **synergizing** proteins that may be used in combination with antifungal CBPs according to the invention include, but are not limited to, .beta.-1,3-**glucanases and chitinases which are obtainable from barley** (Swegle M. et al., 1989, Plant Mol. Biol. 12, 403-412; Balance G. M. et al., 1976, Can. J. Plant Sci. 56, 459-466 Hoj P. B. et al., 1988, FEBS Lett. 230, 67-71; Hoj P. B. et al., 1989, Plant Mol. Biol. 13, 31-42 1989), bean (Boller T. et al, 1983, Planta 157, 22-31; Broglie K. E. et al. 1986, Proc. Natl. Acad. Sci. USA 83, 6820-6824; Vdgeli U. et al., 1988 Planta 174, 364-372); Mauch F. & Staehelin L. A., 1989, Plant-Cell 1, 447-457); cucumber (Metraux J. P. & Boller T. (1986), Physiol. Mol. Plant Pathol. 28, 161-169); leek (Spanu P. et al., 1989, Planta 177, 447-455); maize (Nasser W. et al., 1988, Plant Mol. Biol. 11, 529-538), oat (Fink W. et al., 1988, Plant Physiol. 88, 270-275), pea (Mauch F. et al. 1984, Plant Physiol. 76, 607-611; Mauch F. et al., 1988, Plant Physiol. 87, 325-333), poplar (Parsons, T. J. et al, 1989, P.N.A.S. 86, 7895-7899), potato (Gaynor J. J. 1988, Nucl. Acids Res. 16, 5210; Kombrink E. et al. 1988, Proc. Natl. Acad. Sci. USA 85, 782-786; Laflamme D. and Roxby R., 1989, Plant Mol. Biol. 13, 249-250), tobacco (e.g. Legrand M. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 6750-6754; Shinshi H. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 89-93), tomato (Joosten M. H. A. & De Wit P. J. G. M. 1989, Plant Physiol. 89, 945-951), wheat (Molano J. et al., 1979, J. Biol. Chem. 254, 4901-4907), and the like.

US-PAT-NO: 5981844

DOCUMENT-IDENTIFIER: US 5981844 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 994418

DATE FILED: December 19, 1997

PARENT-CASE:

This Application is a Continuation of U.S. application Ser. No. 08/456,430 filed Jun. 1, 1995, now U.S. Pat. No. 5,703,044, which is a division of Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a Continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a Continuation in-Part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned. Such applications are herein incorporated by reference.

US-CL-CURRENT: 800/301, 435/320.1 , 435/419 , 800/279

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

5 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and **barley AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn AFP preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they **synergized** with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, **synergized** with all AFP preparations, but **synergy** was particularly dramatic with corn-AFP preparations. Polyoxin **synergized** significantly with corn and wheat AFP preparations, while modest **synergy** was observed with combinations of amphotericin and AFP preparations from barley and corn. In contrast, no **synergy** was observed with papulocandin and AFP preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-AFP preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using **synergy** with nikkomycin to inhibit the growth of C albicans as an activity assay, the **synergizing** activity in corn-AFP preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

US-PAT-NO: 5703044

DOCUMENT-IDENTIFIER: US 5703044 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: December 30, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 456430

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional of application Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-CL-CURRENT: 514/12, 514/2 , 514/8 , 530/372 , 530/376

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

26 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and **barley AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn AFP preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they **synergized** with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, **synergized** with all AFP preparations, but **synergy** was particularly dramatic with corn-AFP preparations. Polyoxin **synergized** significantly with corn and wheat AFP preparations, while modest **synergy** was observed with combinations of amphotericin and AFP preparations from barley and corn. In contrast, no **synergy** was observed with papulocandin and AFP preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-AFP preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using **synergy** with nikkomycin to inhibit the growth of C. albicans as an activity assay, the **synergizing** activity in corn-AFP preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

US-PAT-NO: 5670349

DOCUMENT-IDENTIFIER: US 5670349 A

TITLE: HMG2 promoter expression system and post-harvest production of gene products in plants and plant cell cultures

DATE-ISSUED: September 23, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cramer; Carole Lyn	Blacksburg	VA	N/A	N/A
Weissenborn; Deborah Louise	Blacksburg	VA	N/A	N/A

APPL-NO: 08/ 282581

DATE FILED: July 29, 1994

PARENT-CASE:

This application is a continuation-in-part of application Ser. No. 08/100,816 filed Aug. 2, 1993, now abandoned, which is hereby incorporated by reference in its entirety.

US-CL-CURRENT: 435/69.1, 435/320.1 , 536/23.1 , 536/24.1

ABSTRACT:

The invention relates in part to plant HMG2 HMGR genes and in part to the "post-harvest" production method of producing gene product of interest in plant tissues and cultures. The HMG2 promoter elements are responsive to pathogen-infection, pest-infestation, wounding, or elicitor or chemical treatments. The HMG2 elements are also active in specialized tissues of the plant including pollen and mature fruits. HMG2 promoter elements and HMG2-derived promoters can be advantageously used to drive the expression of disease and pest resistance genes, whereby transgenic plants having such gene constructs would be resistant to the targeted disease and pest. In particular, the HMG2 gene expression system can be utilized in developing nematode resistant plants. The post-harvest production method of the invention utilizes plant tissues and cell cultures of plants or plant cells engineered with a expression construct comprising an inducible promoter, such as the HMG2 promoter, operably linked to a gene of interest. Production of the desired gene product is obtained by harvesting, followed by inducing and processing the harvested tissue or culture. The post-harvest production method may be advantageously used to produce direct or indirect gene products that are labile, volatile, toxic, hazardous, etc.

19 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

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Detailed Description Paragraph Table - DETL (1):

TABLE 3

Inducible Plant Genes with Potential for Post-harvest Induction and Accumulation of Transgene Products. An asterisk designates those genes for which promoters have been isolated and characterized. References represent one to two representative references or relevant review article and are not intended to be exhaustive. Genes/gene products Functions Sources of clones

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Defense-Response Genes.sup.a1 Phytoalexin biosynthesis Phenylpropanoid phytoalexin *Phenylalanine ammonia lyase (PAL).sup.2 Enyzme, central pathway Bean, parsley, potato, tomato 4-Coumarate CoA ligase (4CL).sup.3 Enzyme, central pathway Parsley, potato *Chalcone synthase (CHS).sup.4,5 Enzyme, Isoflavanoid branch Bean, soybean, parsley Chalcone isomerase (CHI).sup.6 Enzyme, Isoflavanoid branch Bean Resveratrol (stilbene) synthase.sup.7 Enzyme, Isoflavanoid branch Grapevine, peanut Isoflavone reductase (IFR).sup.8 Enzyme, Isoflavanoid branch Alfalfa Terpenoid phytoalexins *HMG-CoA reductase (HMG).sup.9,10 Enzymes, central pathway Tomato, tobacco, potato, rice Casbene synthetase.sup.11 Casbene biosynthesis Castor bean Cell wall components Lignin *Phenylalanine ammonia lyase See above Cinnamyl alcohol dehydrogenase (CAD).sup.12 Lignin biosyn. Tobacco Caffeic acid o-methyltransferase.sup.13 Lignin biosyn. Alfalfa, tobacco Lignin-forming peroxidase.sup.14 Lignin polymerization Tobacco, wheat Hydroxyproline-rich glycoproteins (HRGP).sup.15,16 Structural protein Bean, tomato Glycine-rich proteins (GRP).sup.15 Structural protein Bean, potato, pea, rice Thionins.sup.17 Antifungal Barley Hydrolases, lytic enzymes *Chitinases (PR-P, PR-Q).sup.18-20 Class I chitinase, basic Vacuolar, antifungal Tobacco, bean, tomato Class I and II chitinase, acidic Extracellular, antifungal Bean Class II chitinase Bifunctional lysozyme, Cucumber, tobacco, **chitinase barley,** petunia *.beta.-1,3-Glucanase.sup.21 Antifungal, chitinase Bean, tobacco, potato, **synergist** pea, rice, Arabidopsis .beta.-fructosidase.sup.22 Antifungal invertase Tomato Others *Proteinase inhibitors (PI-I, PI-II).sup.23,24 Trypsin-, chymotrypsin- Potato, tomato inhibitors Superoxide dismutase (SOD).sup.25 Anti-oxidant exzyme Tobacco, maize, tomato Lipoxygenase.sup.26 Lipid peroxidation, Arabidopsis jasmonate biosyn. Additional "pathogenesis-related" prot. *PR1 family, PR2, PR3.sup.27-29 Unknown Tobacco, bean, parsley, pea Osmotin, PR5.sup.30-32 Antifungal, thaumatin-like Tobacco, maize Ubiquitin.sup.33 Protein degradation Potato Wound-Inducible Genes.sup.a *win1, *win2 (hevein-like).sup.34 Chitin-binding prot. Potato (hevein, rubber tree) wun1, wun2.sup.35 Unknown Potato *nos, nopaline synthase.sup.36 Agrobacterium nutr. Agrobacterium tumefaciens ACC synthase.sup.37 Ethylene biosynthesis Tomato, squash HMG-CoA reductase hmg1.sup.38 Sterol/alkaloid synth. Potato 3-deoxy-D-arabino-heptulosonate- Lignin biosyn. Potato, tomato 7-phosphate

synthase.sup.39 HSP70.sup.33 Heat-shock protein, Potato chaparone Salicylic acid inducible.sup.40 acid peroxidase.sup.14 Lignin-forming Tobacco PR-proteins.sup.40,41 (see above) Tobacco Glycine-rich protein.sup.41 Cell wall protein Tobacco Methyl jasmonate inducible *vspB.sup.42 Vacuolar storage prot. Soybean Proteinase inhibitors I and II.sup.43 Trypsin, chymotrypsin inhib. Potato, tomato Heat-shock genes.sup.43 HSP70.sup.33 Chaperonin Potato Ubiquitin (see above) Cold-stress inducible.sup.44 Drought, salt stress.sup.45 Osmotin.sup.30-32 Desiccation tolerance Tobacco, maize Hormone inducible Gibberellin .alpha.-amylase.sup.46 Starch degradation Barley Abscisic acid.sup.45,47 EM-1, RAB, LEA genes.sup.45 Unknown, embryogenesis Wheat, rice, maize, cotton Ethylene Chitinase, phytoalexin biosyn. genes (see above)

.sup.a Genes are transcriptionally activated in response to pathogens, defense elicitors, wounding and in some cases methyl jasmonate, salicylic acid, HgCl.sub.2 or H.sub.2 O.sub.2. References: .sup.1 Cramer et al., 1993, J. Nematol. 25:507-518. .sup.2 Lois et al., 1989, EMBO J. 8:1641-1648. .sup.3 BeckerAndre et al., 1991, J. Biol. Chem. 266:8551-8559. .sup.4 Arias et al., 1993, Plant Cell 5:485-496. .sup.5 Doerner et al., 1990, Bio/Technology 8:845-848. .sup.6 Mehdy et al., 1987, EMBO J. 6:1527-1533. .sup.7 Hain et al., 1993, Nature 361:153-156. .sup.8 Paiva et al., 1991, Plant Mol. Biol. 17:653-667. .sup.9 Park et al., 1992, Plant Mol. Biol. 20:327-331. .sup.10 Yang et al., 1991, Plant Cell 3:397-405. .sup.11 Lois et al., 1990, Arch. Biochem. Biophys. 276:270-277. .sup.12 Schuch et al., 1991, 3rd Int. Cong. Plant Mol. Biol., Tucson, Az. Abstract 1653. .sup.13 Jaeck et al., 1992, Mol. PlantMicrobe Interact. 5:294-300. .sup.14 Lagrimini et al., 1987, Proc. Natl. Acad. Sci. USA 84:7542-7546. .sup.15 Showalter et al., 1992, Plant Mol. Biol. 19:205-215. .sup.16 Wycoff et al., 1992, in D. P. S. Verma, ed., Molecular Signals in PlantMicrobe Communication, Boca Raton, FL: CRC Press. pgs. 407-422. .sup.17 Bohlmann et al., 1988, EMBO J. 7:1559-1565. .sup.18 Hedrick et al., 1988, Plant Physiol. 86:182-186. .sup.19 Roby et al., 1990, Plant Cell 2:999-1007. .sup.20 Samac and Shah, 1991, Plant Cell 3:1063-1072. .sup.21 Legrand et al., 1987, Proc. Natl. Acad. Sci. USA 84:6750-6754. .sup.22 Benhamou et al., 1991, Plant Physiol. 97:739-750. .sup.23 Ryan, C. A., 1990, Annu. Rev. Phytopathol. 28:425-449. .sup.24 Keil et al., 1989, EMBO J. 8:1323-1330. .sup.25 Bowler et al., 1989, EMBO J. 8:31-38. .sup.26 Melan et al., 1993, Plant Physiol. 101:441-450. .sup.27 Cornelissen et al., 1986, EMBO J. 5:37-40. .sup.28 Meler et al., 1991, Plant Cell 3:309-315. .sup.29 Sharma et al., 1992, Mol. PlantMicrobe Interact. 5:89-95. .sup.30 Cornelissen et al., 1986, Nature 321:531-532. .sup.31 Stinizi et al., 1991, Physiol. Mol. Plant Pathol. 38:137-146. .sup.32 Kononowicz et al., 1992, Plant Cell 4:513-524. .sup.33 Rickey and Belknap, 1991, Plant Mol. Biol. 16:1009-1018. .sup.34 Weiss and Bevan, 1991, Plant Physiol. 96:943-951. .sup.35 Logemann et al., 1988, Proc. Natl. Acad. Sci. USA 85:1136-1140. .sup.36 An et al., 1990, Plant Cell 2:225-233. .sup.37 Li et al., 1992, Plant Mol. Biol. 18:477-487. .sup.38 Chol et al., 1992, Plant Cell 4:1333-1344. .sup.39 Dyer et al., 1989, Proc. Natl. Acad. Sci. USA 86:7370-7373. .sup.40 Ward et al., 1991, Plant Cell 3:1085-1094. .sup.41 Van de Rhee et al., 1990, Plant Cell 2:357-366. .sup.42 Mason et al., 1993, Plant Cell 5:241-251. .sup.43 Ho and Sachs, 1989, In: Stump.sub.-- and Conn, eds., The Biochemistry of Plants: A Comprehensive Treatis, Vol. 15, pgs. 347-377. .sup.44 Thomashow, 1992, In: Scandalios and Wright, eds., Genomic Responses to Environmental Stress, Adv. in Genetics, Vol. 28, pgs. 99-131 .sup.45 Skriver and Mundy, 1990, Plant Cell 2:503-512.

.sup.46 Gubler and Jacobsen, 1992, Plant Cell 4:1435-1441. .sup.47 Chandler and Robertson, 1994, Annu. Rev. Plant Physiol. Plant Mol Biol. 45:113-142.

US-PAT-NO: 5662901

DOCUMENT-IDENTIFIER: US 5662901 A

TITLE: Enzymatic grain conditioner and methods of using it

DATE-ISSUED: September 2, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tobey, Jr.; James F.	Roanoke	VA	N/A	N/A
McGee; J. Stanley	Longmont	CO	N/A	N/A
Cobb; Charles W.	Hereford	TX	N/A	N/A
Cortner; William	Maysville	MO	N/A	N/A

APPL-NO: 08/ 294087

DATE FILED: August 22, 1994

PARENT-CASE:

RELATED APPLICATIONS

This application is a divisional application of pending U.S. patent application Ser. No. 07/544,022 filed Jun. 26, 1990, a continuation of U.S. patent application Ser. No. 07/407,726 filed Sep. 14, 1989 and now abandoned, a continuation of U.S. patent Ser. No. 07/076,114 filed Jul. 21, 1987 and now abandoned.

US-CL-CURRENT: 424/94.2, 424/94.6 , 424/94.61 , 424/94.63 , 426/53 , 426/54 , 426/63 , 435/195 , 435/198 , 435/200 , 435/201 , 435/202 , 435/203 , 435/204 , 435/209 , 435/210 , 435/219

ABSTRACT:

The invention comprises two grain conditioners. The first grain conditioner, which is suitable for use on all grains, comprises a pectinase, a protease, a beta-glucanase and an amylase. The second grain conditioner, which is designed for use on easier-to-digest grains, comprises a pectinase, a beta-glucanase, an amylase and a hemicellulase. The invention also comprises animal feeds which comprise a grain which has been conditioned with one of the grain conditioners of the invention designed to be effective on that grain and methods of increasing the weight gain and feed utilization efficiency of an animal comprising feeding the novel animal feeds of the invention to the animal. The invention further comprises a method of conditioning a grain which comprises providing the grain, contacting the grain with one of the grain conditioners of the invention designed to be effective on that grain and incubating the grain and grain conditioner together for at least about 30 minutes. Finally, there is also provided another method of conditioning a grain comprising providing the grain, scarifying the grain, contacting the

grain with one of the grain conditioners of the invention designed to be effective on that grain and incubating the grain and grain conditioner for at least about 30 minutes.

20 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (9):

U.S. Pat. Nos. 2,988,448 (the '448 patent) and 2,988,449 disclose that using diastatic barley malt as an enzyme supplement for animals feeds containing barley and other fibrous grains results in increased weight gain and feeding efficiency. Both of the patents attribute the increases to the cytolytic enzymes (cytases, gumases (beta-**glucanases) and beta-polyglucosidases) present in the barley** malt. Further, the '448 patent teaches that a combination of diastatic barley malt and fungal enzyme preparations act **synergistically** in increasing the value of barley and other fibrous grains in animal feeds. The mechanism of the action of the enzyme preparations is believed to be due to the action of cytases in these preparations which degrade the non-starch carbohydrate material commonly referred to as gums (beta-glucan).

US-PAT-NO: 5559034

DOCUMENT-IDENTIFIER: US 5559034 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: September 24, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 457552

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional application of Ser. No. 08/178,708, filed Jan. 10, 1994, which is a continuation-in-part of Ser. No. 07,505,781, filed Apr. 6, 1990, now abandoned which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-CL-CURRENT: 435/320.1, 435/252.3 , 435/69.1 , 514/12 , 514/2 , 514/8 , 530/372 , 530/376 , 536/22.1 , 536/23.1 , 536/23.6

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

2 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

--------- KWIC ---------

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and **barley AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn AFP preparations. Growth of the important human pathogen Candida albicanswas found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they **synergized** with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, **synergized** with all AFP preparations, but **synergy** was particularly dramatic with corn-AFP preparations. Polyoxin **synergized** significantly with corn and wheat AFP preparations, while modest **synergy** was observed with combinations of amphotericin and AFP preparations from barley and corn. In contrast, no **synergy** was observed with papulocandin and AFP preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-AFP preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using **synergy** with nikkomycin to inhibit the growth of C. albicans as an activity assay, the **synergizing** activity in corn-AFP preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

US-PAT-NO: 5521153

DOCUMENT-IDENTIFIER: US 5521153 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: May 28, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 178708

DATE FILED: January 10, 1994

PARENT-CASE:

This Application is a continuation-in-part application of U.S. application Ser. No. 07/505,781, filed Apr. 6, 1990, which is a continuation-in-part Application of U.S. application Ser. No. 07/104,755 filed Oct. 2, 1987, both now abandoned. Such applications are herein incorporated by reference .

US-CL-CURRENT: 514/2, 514/12 , 514/8 , 530/372 , 530/376

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

15 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (4):

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Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases from Serratia** marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in glucanase preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified glucanase preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both chitinase and glucanase called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	1607	chitinase$1	USPAT; US-PGPUB	2003/04/25 10:35
2	L2	13553	barley	USPAT; US-PGPUB	2003/04/25 10:35
3	L3	2061	glucanase$1	USPAT; US-PGPUB	2003/04/25 10:39
4	L4	145155	psi or protein adj synthesis adj inhibit$8	USPAT; US-PGPUB	2003/04/25 10:40
5	L5	1545	afp or antifungal adj protein	USPAT; US-PGPUB	2003/04/25 10:45
6	L6	85	1 near6 (serratia or marcesens)	USPAT; US-PGPUB	2003/04/25 10:45
7	L7	30	1 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
8	L8	101	3 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
9	L9	13	4 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
10	L10	25	5 near6 (aspergillus or giganteus)	USPAT; US-PGPUB	2003/04/25 10:47
11	L11	25	(6 and (7 or 8 or 9 or 10)) or (7 and (8 or 9 or 10)) or (8 and (9 or 10)) or (9 and 10)	USPAT; US-PGPUB	2003/04/25 11:08
12	L12	10	(6 or 7 or 8 or 9 or 10) same synerg$	USPAT; US-PGPUB	2003/04/25 11:13
13	L13	30	(6 or 7 or 8 or 9 or 10) same transgen$	USPAT; US-PGPUB	2003/04/25 11:14

PGPUB-DOCUMENT-NUMBER: 20030077640

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030077640 A1

TITLE: Process for producing a marker vaccine against a mammalian virus

PUBLICATION-DATE: April 24, 2003

US-CL-CURRENT: 435/6, 424/186.1 , 800/288 , 800/320.1

APPL-NO: 10/ 246243

DATE FILED: September 18, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	10145969.6	2001DE-10145969.6	September 18, 2001

PGPUB-DOCUMENT-NUMBER: 20020174453

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020174453 A1

TITLE: Production of antibodies in transgenic plastids

PUBLICATION-DATE: November 21, 2002

US-CL-CURRENT: 800/288, 435/320.1 , 530/388.1

APPL-NO: 09/ 807721

DATE FILED: April 18, 2001

PCT-DATA:
APPL-NO: PCT/US01/06274
DATE-FILED: Feb 28, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Application No. 60/185,661, filed Feb. 29, 2000. This application is herein incorporated by reference.

PGPUB-DOCUMENT-NUMBER: 20020174452

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020174452 A1

TITLE: Monocot seeds with increased lignan content

PUBLICATION-DATE: November 21, 2002

US-CL-CURRENT: 800/284

APPL-NO: 09/ 944160

DATE FILED: August 30, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60230632 20000907 US

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit of priority from United States Provisional Patent Application No. 60/230,632, filed Sep. 7, 2000, under 35 U.S.C. .sctn. 119, which is incorporated herein by reference.

PGPUB-DOCUMENT-NUMBER: 20020164798

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164798 A1

TITLE: Process for inducing direct somatic embryogenesis and secondary embryogenesis in monocotyledonous plant cells, and rapidly regenerating fertile plants

PUBLICATION-DATE: November 7, 2002

US-CL-CURRENT: 435/424

APPL-NO: 09/ 929831

DATE FILED: August 14, 2001

RELATED-US-APPL-DATA:

child 09929831 A1 20010814

parent continuation-in-part-of 09641243 20000817 US PENDING

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a Continuation in Part of co-pending U.S. patent application No. 09/641,243, filed Aug. 17, 2000, which is incorporated by reference herein to the extent that there is no inconsistency with the present disclosure.

PGPUB-DOCUMENT-NUMBER: 20020065408

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020065408 A1

TITLE: HMG2 promoter expression system and post-harvest production of gene products in plants and plant cell cultures

PUBLICATION-DATE: May 30, 2002

US-CL-CURRENT: 536/24.1, 536/23.6 , 800/278

APPL-NO: 09/ 902653

DATE FILED: July 12, 2001

RELATED-US-APPL-DATA:

child 09902653 A1 20010712

parent continuation-of 08890624 19970709 US ABANDONED

child 08890624 19970709 US

parent continuation-of 08282581 19940729 US GRANTED

parent-patent 5670349 US

child 08282581 19940729 US

parent continuation-in-part-of 08100816 19930802 US ABANDONED

[0001] This application is a continuation-in-part of co-pending application Ser. No. 08/100,816 filed Aug. 2, 1993, which is hereby incorporated by reference in its entirety.

US-PAT-NO: 6521590

DOCUMENT-IDENTIFIER: US 6521590 B1

TITLE: Biocidal proteins

DATE-ISSUED: February 18, 2003

US-CL-CURRENT: 514/2, 514/16 , 530/300 , 530/328 , 530/350

APPL-NO: 09/ 298574

DATE FILED: April 29, 1999

PARENT-CASE:

This is a continuation of application Ser. No. 08/777,113 filed on Dec. 30, 1996, now U.S. Pat. No. 5,986,176, which is a division of application Ser. No. 08/451,566 filed May 26, 1995, now U.S. Pat. No. 5,691,199, which is a division of application Ser. No. 08/149,839 filed Nov. 10, 1993, now U.S. Pat. No. 5,514,779, which is a continuation-in-part of application Ser. No. 08/002,842 filed Jan. 14, 1993 now abandoned, which is a continuation-in-part of PCT/GB92/00999 filed Jun. 3, 1992.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993
GB	9303564	February 23, 1993

US-PAT-NO: 6521435

DOCUMENT-IDENTIFIER: US 6521435 B1

TITLE: Nucleic acid sequences encoding cell wall-degrading enzymes and use to engineer resistance to Fusarium and other pathogens

DATE-ISSUED: February 18, 2003

US-CL-CURRENT: 435/206, 435/183 , 435/200 , 435/252.3 , 435/320.1 , 435/419 , 435/468 , 435/69.1 , 536/23.2 , 800/295 , 800/298 , 800/320.3

APPL-NO: 09/ 649747

DATE FILED: August 28, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications Nos. 60/224,946, filed Aug. 11, 2000 and 60/151,582, filed Aug. 30, 1999. The disclosure of each of said provisional application is incorporated herein by reference in its entirety.

US-PAT-NO: 6433252

DOCUMENT-IDENTIFIER: US 6433252 B1
See image for Certificate of Correction

TITLE: Maize L3 oleosin promoter

DATE-ISSUED: August 13, 2002

US-CL-CURRENT: 800/287, 435/418 , 435/419 , 435/468 , 536/23.4 , 536/23.6 , 536/23.7 , 536/24.1 , 800/278 , 800/279 , 800/312 , 800/314 , 800/316 , 800/317.2 , 800/317.3 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3

APPL-NO: 09/ 695782

DATE FILED: October 24, 2000

PARENT-CASE:

The present application is a continuation of U.S. Ser. No. 09/080,625, now U.S. Pat. No. 6,307,123 filed May 18, 1998.

US-PAT-NO: 6359196

DOCUMENT-IDENTIFIER: US 6359196 B1

TITLE: Germination-specific plant promoters

DATE-ISSUED: March 19, 2002

US-CL-CURRENT: 800/278, 435/320.1 , 435/419 , 435/6 , 536/23.1 , 536/24.1 , 536/24.3 , 800/288

APPL-NO: 09/ 404390

DATE FILED: September 23, 1999

US-PAT-NO: 6308458

DOCUMENT-IDENTIFIER: US 6308458 B1

TITLE: Herbicide-tolerant plants and methods of controlling the growth of undesired vegetation

DATE-ISSUED: October 30, 2001

US-CL-CURRENT: 504/246, 504/116.1 , 504/240 , 504/243 , 504/265 , 504/281 , 504/283 , 800/300

APPL-NO: 09/ 497698

DATE FILED: February 3, 2000

PARENT-CASE:

This application is a divisional application of U.S. application Ser. No. 09/102,420, filed Jun. 22, 1998, now U.S. Pat. No. 6,084,155, issued Jul. 4, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/059,164, filed Apr. 13, 1998, which is a continuation-in-part of U.S. application Ser. No. 09/050,603, filed Mar. 30, 1998, now U.S. Pat. No. 6,023,012, issued Feb. 8, 2000, which is a continuation-in-part of U.S. application Ser. No. 08/808,931, filed Feb. 28, 1997, now U.S. Pat. No. 5,939,602, issued Aug. 17, 1999, which is a continuation-in-part of U.S. application Ser. No. 08/472,028, filed Jun. 6, 1995, now U.S. Pat. No. 5,767,373, issued Jun. 16, 1998. Said U.S. application Ser. No. 08/808,931 also claims the benefit of U.S. Provisional Application No. 60/012,705, filed on Feb. 28, 1996, U.S. Provisional Application No. 60/013,612, filed on Feb. 28, 1996, and U.S. Provisional Application No. 60/020,003, filed on Jun. 21, 1996. Said U.S. application Ser. No. 09/059,164 also claims the benefit of U.S. Provisional Application No. 60/126,430, filed Mar. 11, 1998. All of the aforementioned applications are incorporated herein by reference.

US-PAT-NO: 6307129

DOCUMENT-IDENTIFIER: US 6307129 B1

TITLE: Herbicide tolerant plants, plant tissue or plant cells having altered protoporphyrinogen oxidase activity

DATE-ISSUED: October 23, 2001

US-CL-CURRENT: 800/300.1, 800/278 , 800/300

APPL-NO: 09/ 191998

DATE FILED: November 12, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation of U.S. application Ser. No. 09/015,683 filed Jan. 29, 1998, which is a Division of U.S. application Ser. No. 08/472,028 filed Jun. 6, 1995, now U.S. Pat. No. 5,767,373, which is a continuation-in-part of U.S. application Ser. No. 08/261,198 filed Jun. 16, 1994, now abandoned.

US-PAT-NO: 6307123

DOCUMENT-IDENTIFIER: US 6307123 B1

TITLE: Methods and compositions for transgene identification

DATE-ISSUED: October 23, 2001

US-CL-CURRENT: 800/282, 536/23.4 , 536/24.1 , 800/266 , 800/288 , 800/300 , 800/301

APPL-NO: 09/ 080625

DATE FILED: May 18, 1998

US-PAT-NO: 6288306

DOCUMENT-IDENTIFIER: US 6288306 B1

TITLE: Methods of selecting plants, plant tissue or plant cells resistant to a protoporphyrinogen oxidase inhibitor

DATE-ISSUED: September 11, 2001

US-CL-CURRENT: 800/300, 435/413 , 435/419 , 800/278

APPL-NO: 09/ 015683

DATE FILED: January 29, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 08/472,028, filed Jun. 6, 1995, now U.S. Pat. No. 5,767,373, issued Jun. 16, 1998, which is a continuation-in-part of U.S. application Ser. No. 08/261,198, filed Jun. 16, 1994, now abandoned.

US-PAT-NO: 6282837

DOCUMENT-IDENTIFIER: US 6282837 B1

TITLE: Methods of controlling the growth of undesired vegetation with herbicide tolerant plants or plant seeds having altered protoporphyrinogen oxidase activity

DATE-ISSUED: September 4, 2001

US-CL-CURRENT: 504/224, 504/243 , 504/285 , 800/300

APPL-NO: 09/ 196268

DATE FILED: November 19, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation of U.S. application Ser. No. 09/015,683 filed Jan. 29, 1998, which is a Division of U.S. application Ser. No. 08/472,028 filed Jun. 6, 1995, now U.S. Pat. No. 5,767,373, which is a continuation-in-part of U.S. application Ser. No. 08/261,198 filed Jun. 16, 1994, now abandoned.

US-PAT-NO: 6277615

DOCUMENT-IDENTIFIER: US 6277615 B1

TITLE: (1.fwdarw.3, 1.fwdarw.4)--.beta.-glucanase of enhanced stability

DATE-ISSUED: August 21, 2001

US-CL-CURRENT: 435/200, 435/69.1 , 530/372

APPL-NO: 08/ 584008

DATE FILED: January 11, 1996

PARENT-CASE:

This application is a continuation-in-part application of PCT/AU94/00377, filed on Jul. 6, 1994, which designated the United States and is entitled to priority under 35 USC .sctn.120.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
AU	PL9821	July 7, 1993

US-PAT-NO: 6271442

DOCUMENT-IDENTIFIER: US 6271442 B1

TITLE: Method of producing pathogen-resistant plants

DATE-ISSUED: August 7, 2001

US-CL-CURRENT: 800/298, 435/252.3 , 435/320.1 , 435/419 , 435/468 , 435/69.1 , 536/23.2 , 536/23.6 , 536/24.1 , 800/278 , 800/295

APPL-NO: 08/ 775362

DATE FILED: January 3, 1997

PARENT-CASE:

This is a continuation of application Ser. No. 08/375,186 filed on Jan. 18, 1995 now U.S. Pat. No. 5,633,442 which is a continuation of Ser. No. 07/810,390 filed Dec. 19, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	40 40 954	December 20, 1990

US-PAT-NO: 6177245

DOCUMENT-IDENTIFIER: US 6177245 B1

TITLE: Manipulation of protoporphyrinogen oxidase enzyme activity in eukaryotic organisms

DATE-ISSUED: January 23, 2001

US-CL-CURRENT: 435/6, 536/23.1 , 536/24.3 , 536/24.31 , 536/24.32

APPL-NO: 09/ 071296

DATE FILED: May 1, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 08/261,198 filed Jun. 16, 1994 now abandoned.

US-PAT-NO: 6137030

DOCUMENT-IDENTIFIER: US 6137030 A
See image for Certificate of Correction

TITLE: Pap mutants that exhibit anti-viral and/or anti-fungal activity in plants

DATE-ISSUED: October 24, 2000

US-CL-CURRENT: 800/279

APPL-NO: 09/ 005273

DATE FILED: January 9, 1998

PARENT-CASE:

This Application is a Continuation of PCT/US96/11546 filed Jul. 11, 1996, which is a Continuation-In-Part of U.S. application Ser. No. 08/500,611 filed Jul. 11, 1995, now U.S. Pat. No. 5,756,322, and application Ser. No. 08/500,694 filed Jul. 11, 1995, now U.S. Pat. No. 5,880,329.

US-PAT-NO: 6084155

DOCUMENT-IDENTIFIER: US 6084155 A

TITLE: Herbicide-tolerant protoporphyrinogen oxidase ("protox") genes

DATE-ISSUED: July 4, 2000

US-CL-CURRENT: 800/300, 435/320.1 , 435/419 , 435/440 , 536/23.2 , 536/23.6 , 800/306 , 800/312 , 800/314 , 800/317.3 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3

APPL-NO: 09/ 102420

DATE FILED: June 22, 1998

PARENT-CASE:

This application is a continuation-in-part of U.S. application Ser. No. 09/059,164, filed Apr. 13, 1998, which is a continuation-in-part of U.S. application Ser. No. 09/050,603, filed Mar. 30, 1998, which is a continuation-in-part of U.S. application Ser. No. 08/808,931, filed Feb. 28, 1997, now U.S. Pat. No. 5,939,602 which is a continuation-in-part of U.S. application Ser. No. 08/472,028, filed Jun. 6, 1995, now U.S. Pat. No. 5,767,373. Said U.S. application Ser. No. 08/808,931 claims the benefit of U.S. Provisional Application No. 60/012,705, filed Feb. 28, 1996, U.S. Provisional Application No. 60/013,612, filed Feb. 28, 1996, and U.S. Provisional Application No. 60/020,003, filed Jun. 21, 1996. Said U.S. application Ser. No. 09/059,164 claims the benefit of U.S. Provisional Application No. 60/126,430, filed Mar. 11, 1998. All of the aforementioned applications are incorporated herein by reference.

US-PAT-NO: 6043415

DOCUMENT-IDENTIFIER: US 6043415 A

TITLE: Synthetic Bacillus thuringiensis cryic gene encoding insect toxin

DATE-ISSUED: March 28, 2000

US-CL-CURRENT: 800/317.3, 435/320.1 , 435/419 , 536/23.71 , 800/279 , 800/302 , 800/317.2 , 800/317.4 , 800/320.1 , 800/322

APPL-NO: 08/ 771986

DATE FILED: December 23, 1996

PARENT-CASE:

This application claims priority to provisional application Ser. No. 60/027,896, filed Oct. 7, 1996, which is incorporated herein by reference.

US-PAT-NO: 5986176

DOCUMENT-IDENTIFIER: US 5986176 A

TITLE: Transgenic plants expressing biocidal proteins

DATE-ISSUED: November 16, 1999

US-CL-CURRENT: 800/301, 800/298

APPL-NO: 08/ 777113

DATE FILED: December 30, 1996

PARENT-CASE:

This is a division of application Ser. No. 08/451,566, filed May 26, 1995, now U.S. Pat. No. 5,691,199 which is a division of application Ser. No. 08/149,839 filed Nov. 10, 1993, now U.S. Pat. No. 5,514,779, which is a continuation-in-part of application Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned, which is a continuation-in-part of PCT/GB92/00999 filed Jun. 3. 1992.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223798	November 12, 1992
GB	9303564	February 23, 1993

US-PAT-NO: 5981835

DOCUMENT-IDENTIFIER: US 5981835 A

TITLE: Transgenic plants as an alternative source of lignocellulosic-degrading enzymes

DATE-ISSUED: November 9, 1999

US-CL-CURRENT: 800/278, 435/410 , 435/414 , 435/468 , 435/469 , 435/69.1 , 435/70.1 , 536/23.1 , 536/23.74 , 800/284 , 800/317.3

APPL-NO: 08/ 883495

DATE FILED: June 26, 1997

PARENT-CASE:

Priority is claimed to provisional application serial No. 60/028,718, filed Oct. 17, 1996.

US-PAT-NO: 5908975

DOCUMENT-IDENTIFIER: US 5908975 A

TITLE: Accumulation of fructans in plants by targeted expression of bacterial levansucrase

DATE-ISSUED: June 1, 1999

US-CL-CURRENT: 800/298, 435/320.1 , 435/419 , 435/468 , 536/23.2 , 536/23.7 , 800/317.2 , 800/317.3 , 800/320.1

APPL-NO: 08/ 640732

DATE FILED: May 6, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is the national stage of international application PCT/US94/12778, filed Nov. 7, 1994, which is a continuation-in-part of U.S. patent application Ser. No. 08/149,689, filed Nov. 9, 1993 now abandoned.

PCT-DATA:
APPL-NO: PCT/US94/12778
DATE-FILED: November 7, 1994
PUB-NO: WO95/13389
PUB-DATE: May 18, 1995
371-DATE: May 6, 1996
102(E)-DATE:May 6, 1996

US-PAT-NO: 5804184

DOCUMENT-IDENTIFIER: US 5804184 A

TITLE: Transgenic pathogen-resistant organism

DATE-ISSUED: September 8, 1998

US-CL-CURRENT: 424/94.61, 424/94.2 , 435/200 , 435/209 , 514/12

APPL-NO: 08/ 812025

DATE FILED: March 6, 1997

PARENT-CASE:

This is a divisional of application No. 08/457,797, filed on Jun. 1, 1995, now U.S. Pat. No. 5,689,045, which is a continuation of Ser. No. 08/134,416, filed on Oct. 8, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 34 131.0	October 9, 1992

US-PAT-NO: 5691199

DOCUMENT-IDENTIFIER: US 5691199 A

TITLE: DNA encoding biocidal proteins

DATE-ISSUED: November 25, 1997

US-CL-CURRENT: 435/325, 435/252.3 , 530/324 , 530/379 , 536/23.6

APPL-NO: 08/ 451566

DATE FILED: May 26, 1995

PARENT-CASE:

This is a division of application Ser. No. 08/149,839, filed Nov. 10 1993, now U.S. Pat. No. 5,514,379 which is a CIP of application Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993

US-PAT-NO: 5670349

DOCUMENT-IDENTIFIER: US 5670349 A

TITLE: HMG2 promoter expression system and post-harvest production of gene products in plants and plant cell cultures

DATE-ISSUED: September 23, 1997

US-CL-CURRENT: 435/69.1, 435/320.1 , 536/23.1 , 536/24.1

APPL-NO: 08/ 282581

DATE FILED: July 29, 1994

PARENT-CASE:

This application is a continuation-in-part of application Ser. No. 08/100,816 filed Aug. 2, 1993, now abandoned, which is hereby incorporated by reference in its entirety.

US-PAT-NO: 5633442

DOCUMENT-IDENTIFIER: US 5633442 A
See image for Certificate of Correction

TITLE: Method of producing pathogen-resistant plants

DATE-ISSUED: May 27, 1997

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 435/488 , 435/69.1 , 536/23.2 , 536/23.6 , 800/301

APPL-NO: 08/ 375186

DATE FILED: January 18, 1995

PARENT-CASE:

This application is a continuation of application Ser. No. 08/215,163, filed on Mar. 21, 1994, now abandoned, which is a continuation of U.S. Pat. No. 07/810,390, filed on Dec. 19, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	40 40 954.6	December 20, 1990

US-PAT-NO: 5597801

DOCUMENT-IDENTIFIER: US 5597801 A

TITLE: Biocidal proteins

DATE-ISSUED: January 28, 1997

US-CL-CURRENT: 514/12, 424/404 , 424/405 , 424/418 , 514/2 , 530/324

APPL-NO: 08/ 451568

DATE FILED: May 26, 1995

PARENT-CASE:

This is a division of application Ser. No. 08/149,839, filed Nov. 10, 1993, which is a CIP of application Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993

US-PAT-NO: 5525716

DOCUMENT-IDENTIFIER: US 5525716 A

TITLE: LPT2 promoter having aleurone-tissue-specific activity

DATE-ISSUED: June 11, 1996

US-CL-CURRENT: 536/24.1, 435/320.1 , 435/69.1 , 435/91.4 , 536/24.3

APPL-NO: 08/ 165315

DATE FILED: December 10, 1993

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9324707	December 2, 1993

US-PAT-NO: 5514779

DOCUMENT-IDENTIFIER: US 5514779 A
See image for Certificate of Correction

TITLE: Biocidal proteins from plants

DATE-ISSUED: May 7, 1996

US-CL-CURRENT: 530/379, 530/300 , 530/324 , 530/333 , 530/350 , 530/370 , 536/23.6

APPL-NO: 08/ 149839

DATE FILED: November 10, 1993

PARENT-CASE:

This application is a Continuation-in-Part of Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993

US-PAT-NO: 6521590

DOCUMENT-IDENTIFIER: US 6521590 B1

TITLE: Biocidal proteins

DATE-ISSUED: February 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Broekaert; Willem Frans	Dilbeek	N/A	N/A	BE
Cammue; Bruno Philippe Angelo	Alsemberg	N/A	N/A	BE
Osborn; Rupert William	Twickenham	N/A	N/A	GB
Rees; Sarah Bronwen	Forest Park	N/A	N/A	GB
Vanderleyden; Jozef	Heverlee	N/A	N/A	BE

APPL-NO: 09/ 298574

DATE FILED: April 29, 1999

PARENT-CASE:

This is a continuation of application Ser. No. 08/777,113 filed on Dec. 30, 1996, now U.S. Pat. No. 5,986,176, which is a division of application Ser. No. 08/451,566 filed May 26, 1995, now U.S. Pat. No. 5,691,199, which is a division of application Ser. No. 08/149,839 filed Nov. 10, 1993, now U.S. Pat. No. 5,514,779, which is a continuation-in-part of application Ser. No. 08/002,842 filed Jan. 14, 1993 now abandoned, which is a continuation-in-part of PCT/GB92/00999 filed Jun. 3, 1992.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993
GB	9303564	February 23, 1993

US-CL-CURRENT: 514/2, 514/16 , 530/300 , 530/328 , 530/350

ABSTRACT:

Biocidal proteins capable of isolation from seeds have been characterized. The proteins have an amino acid sequence containing the common cysteine/glycine domain of Chitin-binding Plant Proteins but show substantially better activity against pathogenic fungi, a higher ratio of basic amino acids to acidic amino acids, and/or antifungal activity which results in increased hyphal branching. Antimicrobial proteins isolated from Amáranthus, Capsicum, Briza and related species are provided. The proteins show a wide range of antifungal activity

and are active against Gram-positive bacteria. DNA encoding the proteins may be isolated and incorporated into vectors. Plants may be transformed with this DNA. The proteins find agricultural or pharmaceutical application as antifungal or antibacterial agents. Transgenic plants expressing the protein will show increased disease resistance.

8 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Brief Summary Text - BSTX (13):

Application of Chitin-binding Plant Proteins, especially chitinases, in the protection of plants against fungal disease has been reported, and the potential usefulness of these proteins to engineer resistance in plants has been described (for example, Pioneer Hi Bred's European Patent Application 502718). In U.S. Pat. No. 4,940,840 (DNA Plant Technology Corporation), tobacco plants expressing a **chitinase gene from the bacterium Serratia** marcescens appear to be less sensitive to the fungus Alternaria longipes. European Patent Application Number 418695 (Ciba Geigy) describes the use of regulatory DNA sequences from tobacco chitinase gene to drive expression of introduced genes producing **transgenic** plants with improved resistance to pathogens. Patent Application Number WO9007001 (Du Pont de Nemours Company) describes production of **transgenic** plants which over-express a chitinase gene giving improved resistance to fungal pathogens.

US-PAT-NO: 6271442

DOCUMENT-IDENTIFIER: US 6271442 B1

TITLE: Method of producing pathogen-resistant plants

DATE-ISSUED: August 7, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schell; Jeff	Koln	N/A	N/A	DE
Logemann; Jurgen	Koln	N/A	N/A	DE
Jach; Guido	Koln	N/A	N/A	DE
Mundy; John	Copenhagen	N/A	N/A	DK

APPL-NO: 08/ 775362

DATE FILED: January 3, 1997

PARENT-CASE:

This is a continuation of application Ser. No. 08/375,186 filed on Jan. 18, 1995 now U.S. Pat. No. 5,633,442 which is a continuation of Ser. No. 07/810,390 filed Dec. 19, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	40 40 954	December 20, 1990

US-CL-CURRENT: 800/298, 435/252.3 , 435/320.1 , 435/419 , 435/468 , 435/69.1 , 536/23.2 , 536/23.6 , 536/24.1 , 800/278 , 800/295

ABSTRACT:

Described are a method of producing pathogen-resistant plants in which a protein-synthesis inhibitor gene or a fusion product of the protein-synthesis inhibitor gene or of the protein-synthesis inhibitor protein with ligands permitting specific attachment to cells is introduced into the genotype of plants under the control of an active promotor, and the use of the protein-synthesis inhibitor protein obtained by introducing the protein-synthesis inhibitor gene into the bacterial overproducers for making pharmaceutical preparations.

17 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Brief Summary Text - BSTX (9):

It has further been found that **PSI genes isolated for example from barley** plants can be fused with a great variety of active promotors, for example the wun1-promotor, which is described in detail in "The Plant Cell 1", 1989, p.151-158 and that such promotor gene fusions can be incorporated into the genotype of plants and can produce **transgenic** plants which exhibit newly acquired pathogenic resistance.

US-PAT-NO: 5986176

DOCUMENT-IDENTIFIER: US 5986176 A

TITLE: Transgenic plants expressing biocidal proteins

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Broekaert; Willem Frans	Dilbeek	N/A	N/A	BE
Cammue; Bruno Phillippe	Alsemberg	N/A	N/A	BE
Angelo	Forest Park	N/A	N/A	GB
Rees; Sarah Bronwen	Heverlee	N/A	N/A	BE
Vanderleyden; Jozef				

APPL-NO: 08/ 777113

DATE FILED: December 30, 1996

PARENT-CASE:

This is a division of application Ser. No. 08/451,566, filed May 26, 1995, now U.S. Pat. No. 5,691,199 which is a division of application Ser. No. 08/149,839 filed Nov. 10, 1993, now U.S. Pat. No. 5,514,779, which is a continuation-in-part of application Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned, which is a continuation-in-part of PCT/GB92/00999 filed Jun. 3. 1992.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223798	November 12, 1992
GB	9303564	February 23, 1993

US-CL-CURRENT: 800/301, 800/298

ABSTRACT:

Biocidal proteins capable of isolation from seeds have been characterized. The proteins have an amino acid sequence containing the common cysteine/glycine domain of Chitin-binding Plant Proteins but show substantially better activity against pathogenic fungi, a higher ratio of basic amino acids to acidic amino acids, and/or antifungal activity which results in increased hyphal branching. Antimicrobial proteins isolated from Amaranthus, Capsicum, Briza and related species are provided. The proteins show a wide range of antifungal activity and are active against Gram-positive bacteria. DNA encoding the proteins may be isolated and incorporated into vectors. Plants may be transformed with this DNA. The proteins find agricultural or pharmaceutical application as antifungal or antibacterial agents. Transgenic plants expressing the protein

will show increased disease resistance.

20 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (15):

Application of Chitin-binding Plant Proteins, especially chitinases, in the protection of plants against fungal disease has been reported, and the potential usefulness of these proteins to engineer resistance in plants has been described (for example, Pioneer Hi Bred's European Patent Application 502718). In U.S. Pat. No. 4,940,840 (DNA Plant Technology Corporation), tobacco plants expressing a **chitinase gene from the bacterium Serratia** marcescens appear to be less sensitive to the fungus Alternaria longipes. European Patent Application Number 418695 (Ciba Geigy) describes the use of regulatory DNA sequences from tobacco chitinase gene to drive expression of introduced genes producing **transgenic** plants with improved resistance to pathogens. Patent Application Number W09007001 (Du Pont de Nemours Company) describes production of **transgenic** plants which over-express a chitinase gene giving improved resistance to fungal pathogens.

US-PAT-NO: 5908975

DOCUMENT-IDENTIFIER: US 5908975 A

TITLE: Accumulation of fructans in plants by targeted expression of bacterial levansucrase

DATE-ISSUED: June 1, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Caimi; Perry Gerard	Landenberg	PA	N/A	N/A
Hershey; Howard Paul	West Chester	PA	N/A	N/A
Kerr; Phillip S.	Urbandale	IA	N/A	N/A

APPL-NO: 08/ 640732

DATE FILED: May 6, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is the national stage of international application PCT/US94/12778, filed Nov. 7, 1994, which is a continuation-in-part of U.S. patent application Ser. No. 08/149,689, filed Nov. 9, 1993 now abandoned.

PCT-DATA:
APPL-NO: PCT/US94/12778
DATE-FILED: November 7, 1994
PUB-NO: WO95/13389
PUB-DATE: May 18, 1995
371-DATE: May 6, 1996
102(E)-DATE:May 6, 1996

US-CL-CURRENT: 800/298, 435/320.1 , 435/419 , 435/468 , 536/23.2 , 536/23.7 , 800/317.2 , 800/317.3 , 800/320.1

ABSTRACT:

This invention concerns methods for synthesis and accumulation of fructose polymers in seed, tubers or leaves of transgenic plants by selective expression of a bacterial fructosyltransferase gene. Selective expression includes coordination of timing, tissue specific expression and especially subcellular location. Successful transformants utilize sucrose to synthesize and accumulate fructan in the vacuole of the cell, in established crops, without loss of co-products or concern for yield loss due to degradation during maturation, harvest or storage of the plant. Enhanced fructan production will benefit the fructose sweetener industry and add value to grain used for feed.

11 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (124):

Vacuolar specific proteins have been demonstrated to be correctly targeted to the vacuole in heterologous **transgenic** plants (Bednarak et al., Plant Cell, 2:1145-1155 (1990); Matsuoka and Nakamura, Proc. Natl. Acad. Sci. 88:834-838 (1991); Holwerda et al., Plant Cell, 4:307-318 (1992)). Demonstration of correct assembly, processing and targeting of the vacuole specific barley lectin protein in tobacco, indicates that the sorting machinery in monocots and dicots is very similar (Wilkins et al., Plant Cell 2:301-313 (1992)). Furthermore, targeting sequences from vacuole specific genes, operably fused to heterologous coding sequences in chimeric gene constructs, also maintain the vacuole specific expression in **transgenic** plants. Such examples include the patatin vacuole targeting sequence fused to the yeast invertase Suc2 gene and established to be correctly targeted to the vacuole of **transgenic** tobacco cells (Sonnewald et al., The Plant J. 1:95-106 (1991)), the C-terminal vacuole targeting sequences of either tobacco chitinase A or in a separate experiment, the C-terminal sequence from **barley lectin were fused to the secreted form of cucumber chitinase**. The chimeric cucumber chitinase was correctly targeted to the vacuole of tobacco cells in both experiments (Neuhaus et al., Proc. Natl. Acad. Sci., 88:10362-10366 (1991); Bednarek and Raikhel, The Plant Cell, 3:1195-1206 (1991)). The source of the vacuole targeting sequence chosen to fuse operationally to the FTF protein is not critical so long as it is sufficient to accomplish the invention by correct targeting of a functional FTF protein to the vacuole of preferred **transgenic** plant cells.

US-PAT-NO: 5691199

DOCUMENT-IDENTIFIER: US 5691199 A

TITLE: DNA encoding biocidal proteins

DATE-ISSUED: November 25, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Broekaert; Willem Frans	Dilbeek	N/A	N/A	BE
Cammue; Bruno Philippe Angelo	Alsemberg	N/A	N/A	BE
Rees; Sarah Bronwen	Berkshire	N/A	N/A	GB2
Vanderleyden; Jozef	Heverlee	N/A	N/A	BE

APPL-NO: 08/ 451566

DATE FILED: May 26, 1995

PARENT-CASE:

This is a division of application Ser. No. 08/149,839, filed Nov. 10 1993, now U.S. Pat. No. 5,514,379 which is a CIP of application Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993

US-CL-CURRENT: 435/325, 435/252.3 , 530/324 , 530/379 , 536/23.6

ABSTRACT:

Biocidal proteins capable of isolation from seeds have been characterized. The proteins have an amino acid sequence containing the common cysteine/glycine domain of Chitin-binding Plant Proteins but show substantially better activity against pathogenic fungi, a higher ratio of basic amino acids to acidic amino acids, and/or antifungal activity which results in increased hyphal branching. Antimicrobial proteins isolated from Amaranthus, Capsicum, Briza and related species are provided. The proteins show a wide range of antifungal activity and are active against Gram-positive bacteria. DNA encoding the proteins may be isolated and incorporated into vectors. Plants may be transformed with this DNA. The proteins find agricultural or pharmaceutical application as antifungal or antibacterial agents. Transgenic plants expressing the protein will show increased disease resistance.

60 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (12):

Application of Chitin-binding Plant Proteins, especially chitinases, in the protection of plants against fungal disease has been reported, and the potential usefulness of these proteins to engineer resistance in plants has been described (for example, Pioneer Hi Bred's European Patent Application 502718). In U.S. Pat. No. 4,940,840 (DNA Plant Technology Corporation), tobacco plants expressing a **chitinase genes from the bacterium Serratia** marcescens appear to be less sensitive to the fungus Alternaria longipes. European Patent Application Number 418695 (Ciba Geigy) describes the use of regulatory DNA sequences from tobacco chitinase genes to drive expression of introduced genes producing **transgenic** plants with improved resistance to pathogens. Patent Application Number WO9007001 (Du Pont de Nemours Company) describes production of **transgenic** plants which over-express a chitinase gene giving improved resistance to fungal pathogens.

US-PAT-NO: 5670349

DOCUMENT-IDENTIFIER: US 5670349 A

TITLE: HMG2 promoter expression system and post-harvest production of gene products in plants and plant cell cultures

DATE-ISSUED: September 23, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cramer; Carole Lyn	Blacksburg	VA	N/A	N/A
Weissenborn; Deborah Louise	Blacksburg	VA	N/A	N/A

APPL-NO: 08/ 282581

DATE FILED: July 29, 1994

PARENT-CASE:

This application is a continuation-in-part of application Ser. No. 08/100,816 filed Aug. 2, 1993, now abandoned, which is hereby incorporated by reference in its entirety.

US-CL-CURRENT: 435/69.1, 435/320.1 , 536/23.1 , 536/24.1

ABSTRACT:

The invention relates in part to plant HMG2 HMGR genes and in part to the "post-harvest" production method of producing gene product of interest in plant tissues and cultures. The HMG2 promoter elements are responsive to pathogen-infection, pest-infestation, wounding, or elicitor or chemical treatments. The HMG2 elements are also active in specialized tissues of the plant including pollen and mature fruits. HMG2 promoter elements and HMG2-derived promoters can be advantageously used to drive the expression of disease and pest resistance genes, whereby transgenic plants having such gene constructs would be resistant to the targeted disease and pest. In particular, the HMG2 gene expression system can be utilized in developing nematode resistant plants. The post-harvest production method of the invention utilizes plant tissues and cell cultures of plants or plant cells engineered with a expression construct comprising an inducible promoter, such as the HMG2 promoter, operably linked to a gene of interest. Production of the desired gene product is obtained by harvesting, followed by inducing and processing the harvested tissue or culture. The post-harvest production method may be advantageously used to produce direct or indirect gene products that are labile, volatile, toxic, hazardous, etc.

19 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (1):

TABLE 3

Inducible Plant Genes with Potential for Post-harvest Induction and Accumulation of **Transgene** Products. An asterisk designates those genes for which promoters have been isolated and characterized. References represent one to two representative references or relevant review article and are not intended to be exhaustive. Genes/gene products Functions Sources of clones

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Defense-Response Genes.sup.a1 Phytoalexin biosynthesis Phenylpropanoid phytoalexin *Phenylalanine ammonia lyase (PAL).sup.2 Enyzme, central pathway Bean, parsley, potato, tomato 4-Coumarate CoA ligase (4CL).sup.3 Enzyme, central pathway Parsley, potato *Chalcone synthase (CHS).sup.4,5 Enzyme, Isoflavanoid branch Bean, soybean, parsley Chalcone isomerase (CHI).sup.6 Enzyme, Isoflavanoid branch Bean Resveratrol (stilbene) synthase.sup.7 Enzyme, Isoflavanoid branch Grapevine, peanut Isoflavone reductase (IFR).sup.8 Enzyme, Isoflavanoid branch Alfalfa Terpenoid phytoalexins *HMG-CoA reductase (HMG).sup.9,10 Enzymes, central pathway Tomato, tobacco, potato, rice Casbene synthetase.sup.11 Casbene biosynthesis Castor bean Cell wall components Lignin *Phenylalanine ammonia lyase See above Cinnamyl alcohol dehydrogenase (CAD).sup.12 Lignin biosyn. Tobacco Caffeic acid o-methyltransferase.sup.13 Lignin biosyn. Alfalfa, tobacco Lignin-forming peroxidase.sup.14 Lignin polymerization Tobacco, wheat Hydroxyproline-rich glycoproteins (HRGP).sup.15,16 Structural protein Bean, tomato Glycine-rich proteins (GRP).sup.15 Structural protein Bean, potato, pea, rice Thionins.sup.17 Antifungal Barley Hydrolases, lytic enzymes *Chitinases (PR-P, PR-Q).sup.18-20 Class I chitinase, basic Vacuolar, antifungal Tobacco, bean, tomato Class I and II chitinase, acidic Extracellular, antifungal Bean Class II chitinase Bifunctional lysozyme, Cucumber, tobacco, **chitinase barley,** petunia *.beta.-1,3-Glucanase.sup.21 Antifungal, chitinase Bean, tobacco, potato, synergist pea, rice, Arabidopsis .beta.-fructosidase.sup.22 Antifungal invertase Tomato Others *Proteinase inhibitors (PI-I, PI-II).sup.23,24 Trypsin-, chymotrypsin- Potato, tomato inhibitors Superoxide dismutase (SOD).sup.25 Anti-oxidant exzyme Tobacco, maize, tomato Lipoxygenase.sup.26 Lipid peroxidation, Arabidopsis jasmonate biosyn. Additional "pathogenesis-related" prot. *PR1 family, PR2, PR3.sup.27-29 Unknown Tobacco, bean, parsley, pea Osmotin, PR5.sup.30-32 Antifungal, thaumatin-like Tobacco, maize Ubiquitin.sup.33 Protein degradation Potato Wound-Inducible Genes.sup.a *win1, *win2 (hevein-like).sup.34 Chitin-binding prot. Potato (hevein, rubber tree) wun1, wun2.sup.35 Unknown Potato *nos, nopaline synthase.sup.36 Agrobacterium nutr. Agrobacterium tumefaciens ACC synthase.sup.37 Ethylene biosynthesis Tomato, squash HMG-CoA reductase hmg1.sup.38 Sterol/alkaloid synth. Potato 3-deoxy-D-arabino-heptulosonate- Lignin biosyn. Potato, tomato 7-phosphate

synthase.sup.39 HSP70.sup.33 Heat-shock protein, Potato chaparone Salicylic acid inducible.sup.40 acid peroxidase.sup.14 Lignin-forming Tobacco PR-proteins.sup.40,41 (see above) Tobacco Glycine-rich protein.sup.41 Cell wall protein Tobacco Methyl jasmonate inducible *vspB.sup.42 Vacuolar storage prot. Soybean Proteinase inhibitors I and II.sup.43 Trypsin, chymotrypsin inhib. Potato, tomato Heat-shock genes.sup.43 HSP70.sup.33 Chaperonin Potato Ubiquitin (see above) Cold-stress inducible.sup.44 Drought, salt stress.sup.45 Osmotin.sup.30-32 Desiccation tolerance Tobacco, maize Hormone inducible Gibberellin .alpha.-amylase.sup.46 Starch degradation Barley Abscisic acid.sup.45,47 EM-1, RAB, LEA genes.sup.45 Unknown, embryogenesis Wheat, rice, maize, cotton Ethylene Chitinase, phytoalexin biosyn. genes (see above)

.sup.a Genes are transcriptionally activated in response to pathogens, defense elicitors, wounding and in some cases methyl jasmonate, salicylic acid, HgCl.sub.2 or H.sub.2 O.sub.2. References: .sup.1 Cramer et al., 1993, J. Nematol. 25:507-518. .sup.2 Lois et al., 1989, EMBO J. 8:1641-1648. .sup.3 BeckerAndre et al., 1991, J. Biol. Chem. 266:8551-8559. .sup.4 Arias et al., 1993, Plant Cell 5:485-496. .sup.5 Doerner et al., 1990, Bio/Technology 8:845-848. .sup.6 Mehdy et al., 1987, EMBO J. 6:1527-1533. .sup.7 Hain et al., 1993, Nature 361:153-156. .sup.8 Paiva et al., 1991, Plant Mol. Biol. 17:653-667. .sup.9 Park et al., 1992, Plant Mol. Biol. 20:327-331. .sup.10 Yang et al., 1991, Plant Cell 3:397-405. .sup.11 Lois et al., 1990, Arch. Biochem. Biophys. 276:270-277. .sup.12 Schuch et al., 1991, 3rd Int. Cong. Plant Mol. Biol., Tucson, Az. Abstract 1653. .sup.13 Jaeck et al., 1992, Mol. PlantMicrobe Interact. 5:294-300. .sup.14 Lagrimini et al., 1987, Proc. Natl. Acad. Sci. USA 84:7542-7546. .sup.15 Showalter et al., 1992, Plant Mol. Biol. 19:205-215. .sup.16 Wycoff et al., 1992, in D. P. S. Verma, ed., Molecular Signals in PlantMicrobe Communication, Boca Raton, FL: CRC Press. pgs. 407-422. .sup.17 Bohlmann et al., 1988, EMBO J. 7:1559-1565. .sup.18 Hedrick et al., 1988, Plant Physiol. 86:182-186. .sup.19 Roby et al., 1990, Plant Cell 2:999-1007. .sup.20 Samac and Shah, 1991, Plant Cell 3:1063-1072. .sup.21 Legrand et al., 1987, Proc. Natl. Acad. Sci. USA 84:6750-6754. .sup.22 Benhamou et al., 1991, Plant Physiol. 97:739-750. .sup.23 Ryan, C. A., 1990, Annu. Rev. Phytopathol. 28:425-449. .sup.24 Keil et al., 1989, EMBO J. 8:1323-1330. .sup.25 Bowler et al., 1989, EMBO J. 8:31-38. .sup.26 Melan et al., 1993, Plant Physiol. 101:441-450. .sup.27 Cornelissen et al., 1986, EMBO J. 5:37-40. .sup.28 Meler et al., 1991, Plant Cell 3:309-315. .sup.29 Sharma et al., 1992, Mol. PlantMicrobe Interact. 5:89-95. .sup.30 Cornelissen et al., 1986, Nature 321:531-532. .sup.31 Stinizi et al., 1991, Physiol. Mol. Plant Pathol. 38:137-146. .sup.32 Kononowicz et al., 1992, Plant Cell 4:513-524. .sup.33 Rickey and Belknap, 1991, Plant Mol. Biol. 16:1009-1018. .sup.34 Weiss and Bevan, 1991, Plant Physiol. 96:943-951. .sup.35 Logemann et al., 1988, Proc. Natl. Acad. Sci. USA 85:1136-1140. .sup.36 An et al., 1990, Plant Cell 2:225-233. .sup.37 Li et al., 1992, Plant Mol. Biol. 18:477-487. .sup.38 Chol et al., 1992, Plant Cell 4:1333-1344. .sup.39 Dyer et al., 1989, Proc. Natl. Acad. Sci. USA 86:7370-7373. .sup.40 Ward et al., 1991, Plant Cell 3:1085-1094. .sup.41 Van de Rhee et al., 1990, Plant Cell 2:357-366. .sup.42 Mason et al., 1993, Plant Cell 5:241-251. .sup.43 Ho and Sachs, 1989, In: Stump.sub.-- and Conn, eds., The Biochemistry of Plants: A Comprehensive Treatis, Vol. 15, pgs. 347-377. .sup.44 Thomashow, 1992, In: Scandalios and Wright, eds., Genomic Responses to Environmental Stress, Adv. in Genetics, Vol. 28, pgs. 99-131 .sup.45 Skriver and Mundy, 1990, Plant Cell 2:503-512.

.sup.46 Gubler and Jacobsen, 1992, Plant Cell 4:1435-1441. .sup.47 Chandler and Robertson, 1994, Annu. Rev. Plant Physiol. Plant Mol Biol. 45:113-142.

US-PAT-NO: 5633442

DOCUMENT-IDENTIFIER: US 5633442 A
See image for Certificate of Correction

TITLE: Method of producing pathogen-resistant plants

DATE-ISSUED: May 27, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schell; Jeff	Koln	N/A	N/A	DE
Logemann; Jurgen	Koln	N/A	N/A	DE
Jach; Guido	Koln	N/A	N/A	DE
Mundy; John	Copenhagen	N/A	N/A	DK

APPL-NO: 08/ 375186

DATE FILED: January 18, 1995

PARENT-CASE:

This application is a continuation of application Ser. No. 08/215,163, filed on Mar. 21, 1994, now abandoned, which is a continuation of U.S. Pat. No. 07/810,390, filed on Dec. 19, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	40 40 954.6	December 20, 1990

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 435/488 , 435/69.1 , 536/23.2 , 536/23.6 , 800/301

ABSTRACT:

Described are a method of producing pathogen-resistant plants in which a protein-synthesis inhibitor gene or a fusion product of the protein-synthesis inhibitor gene or of the protein-synthesis inhibitor protein with ligands permitting specific attachment to cells is introduced into the genotype of plants under the control of an active promotor, and the use of the protein-synthesis inhibitor protein obtained by introducing the protein-synthesis inhibitor gene into the bacterial overproducers for making pharmaceutical preparations.

6 Claims, 14 Drawing figures

Exemplary Claim Number: 1,2,3

Number of Drawing Sheets: 12

---------- KWIC ---------

Brief Summary Text - BSTX (9):

It has further been found that **PSI genes isolated for example from barley** plants can be fused with a great variety of active promotors, for example the wun1-promotor, which is described in detail in "The Plant Cell 1", 1989, p.151-158 and that such promotor gene fusions can be incorporated into the genotype of plants and can produce **transgenic** plants which exhibit newly acquired pathogenic resistance.

US-PAT-NO: 5597801

DOCUMENT-IDENTIFIER: US 5597801 A

TITLE: Biocidal proteins

DATE-ISSUED: January 28, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Broekaert; Willem F.	Dilbeek	N/A	N/A	BE
Cammue; Bruno P. A.	Alsemberg	N/A	N/A	BE
Rees; Sarah B.	Forest Park	N/A	N/A	GB2
Vanderleyden; Jozef	Heverlee	N/A	N/A	BE

APPL-NO: 08/ 451568

DATE FILED: May 26, 1995

PARENT-CASE:

This is a division of application Ser. No. 08/149,839, filed Nov. 10, 1993, which is a CIP of application Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993

US-CL-CURRENT: 514/12, 424/404 , 424/405 , 424/418 , 514/2 , 530/324

ABSTRACT:

Biocidal proteins capable of isolation from seeds have been characterized. The proteins have an amino acid sequence containing the common cysteine/glycine domain of Chitin-binding Plant Proteins but show substantially better activity against pathogenic fungi, a higher ratio of basic amino acids to acidic amino acids, and/or antifungal activity which results in increased hyphal branching. Antimicrobial proteins isolated from Amaranthus, Capsicum, Briza and related species are provided. The proteins show a wide range of antifungal activity and are active against Gram-positive bacteria. DNA encoding the proteins may be isolated and incorporated into vectors. Plants may be transformed with this DNA. The proteins find agricultural or pharmaceutical application as antifungal or antibacterial agents. Transgenic plants expressing the protein will show increased disease resistance.

25 Claims, 29 Drawing figures

Exemplary Claim Number: 3

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (13):

Application of Chitin-binding Plant Proteins, especially chitinases, in the protection of plants against fungal disease has been reported, and the potential usefulness of these proteins to engineer resistance in plants has been described (for example, Pioneer Hi Bred's European Patent Application 502718). In U.S. Pat. No. 4,940,840 (DNA Plant Technology Corporation), tobacco plants expressing a **chitinase gene from the bacterium Serratia** marcescens appear to be less sensitive to the fungus Alternaria longipes. European Patent Application Number 418695 (Ciba Geigy) describes the use of regulatory DNA sequences from tobacco chitinase gene to drive expression of introduced genes producing **transgenic** plants with improved resistance to pathogens. U.S. patent application No. WO9007001 (Du Pont de Nemours Company) describes production of **transgenic** plants which over-express a chitinase gene giving improved resistance to fungal pathogens.

US-PAT-NO: 5514779

DOCUMENT-IDENTIFIER: US 5514779 A
See image for Certificate of Correction

TITLE: Biocidal proteins from plants

DATE-ISSUED: May 7, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Broekaert; Willem F.	Dilbeek	N/A	N/A	BE
Cammue; Bruno P. A.	Alsemberg	N/A	N/A	BE
Rees; Sarah B.	Forest Park	N/A	N/A	GB2
Vanderleyden; Jozef	Heverlee	N/A	N/A	BE

APPL-NO: 08/ 149839

DATE FILED: November 10, 1993

PARENT-CASE:

This application is a Continuation-in-Part of Ser. No. 08/002,842, filed Jan. 14, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9112300	June 7, 1991
GB	9223708	November 12, 1992
GB	9303564	February 23, 1993

US-CL-CURRENT: 530/379, 530/300 , 530/324 , 530/333 , 530/350 , 530/370 , 536/23.6

ABSTRACT:

Biocidal proteins capable of isolation from seeds have been characterized. The proteins have an amino acid sequence containing the common cysteine/glycine domain of Chitin-binding Plant Proteins but show substantially better activity against pathogenic fungi, a higher ratio of basic amino acids to acidic amino acids, and/or antifungal activity which results in increased hyphal branching. Antimicrobial proteins isolated from Amaranthus, Capsicum, Briza and related species are provided. The proteins show a wide range of antifungal activity and are active against Gram-positive bacteria. DNA encoding the proteins may be isolated and incorporated into vectors. Plants may be transformed with this DNA. The proteins find agricultural or pharmaceutical application as antifungal or antibacterial agents. Transgenic plants expressing the protein will show increased disease resistance.

12 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (12):

Application of Chitin-binding Plant Proteins, especially chitinases, in the protection of plants against fungal disease has been reported, and the potential usefulness of these proteins to engineer resistance in plants has been described (for example, Pioneer Hi Bred's European Patent Application 502718). In U.S. Pat. No. 4,940,840 (DNA Plant Technology Corporation), tobacco plants expressing a **chitinase gene from the bacterium Serratia** marcescens appear to be less sensitive to the fungus Alternaria longipes. European Patent Application Number 418695 (Ciba Geigy) describes the use of regulatory DNA sequences from tobacco chitinase gene to drive expression of introduced genes producing **transgenic** plants with improved resistance to pathogens. Patent Application Number WO9007001 (Du Pont de Nemours Company) describes production of **transgenic** plants which over-express a chitinase gene giving improved resistance to fungal pathogens.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	1607	chitinase$1	USPAT; US-PGPUB	2003/04/25 10:35
2	L2	13553	barley	USPAT; US-PGPUB	2003/04/25 10:35
3	L3	2061	glucanase$1	USPAT; US-PGPUB	2003/04/25 10:39
4	L4	145155	psi or protein adj synthesis adj inhibit$8	USPAT; US-PGPUB	2003/04/25 10:40
5	L5	1545	afp or antifungal adj protein	USPAT; US-PGPUB	2003/04/25 10:45
6	L6	85	1 near6 (serratia or marcesens)	USPAT; US-PGPUB	2003/04/25 10:45
7	L7	30	1 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
8	L8	101	3 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
9	L9	13	4 near6 2	USPAT; US-PGPUB	2003/04/25 10:46
10	L10	25	5 near6 (aspergillus or giganteus)	USPAT; US-PGPUB	2003/04/25 10:47
11	L11	25	(6 and (7 or 8 or 9 or 10)) or (7 and (8 or 9 or 10)) or (8 and (9 or 10)) or (9 and 10)	USPAT; US-PGPUB	2003/04/25 11:46
12	L12	10	(6 or 7 or 8 or 9 or 10) same synerg$	USPAT; US-PGPUB	2003/04/25 11:13
13	L13	30	(6 or 7 or 8 or 9 or 10) same transgen$	USPAT; US-PGPUB	2003/04/25 14:19
14	L14	793	(1 same (3 or 4 or 5)) or (3 same (4 or 5)) or (4 same 5)	USPAT; US-PGPUB	2003/04/25 11:48
15	L15	39	14 same synerg$	USPAT; US-PGPUB	2003/04/25 11:49
16	L16	114	14 same transgen$	USPAT; US-PGPUB	2003/04/25 14:19

PGPUB-DOCUMENT-NUMBER: 20030026797

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030026797 A1

TITLE: Antifungal compositions

PUBLICATION-DATE: February 6, 2003

US-CL-CURRENT: 424/94.61, 424/94.63 , 504/117 , 504/129

APPL-NO: 10/ 160886

DATE FILED: May 30, 2002

RELATED-US-APPL-DATA:

child 10160886 A1 20020530

parent continuation-of 09202056 19981207 US PENDING

child 09202056 19981207 US

parent a-371-of-international PCT/EP97/03046 19970609 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	96201651.5	1996EP-96201651.5	June 7, 1996

PGPUB-DOCUMENT-NUMBER: 20020168735

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168735 A1

TITLE: ANTIFUNGAL PROTEINS, DNA CODING THEREFORE, AND HOSTS INCORPORATING SAME

PUBLICATION-DATE: November 14, 2002

US-CL-CURRENT: 435/183, 435/320.1 , 435/325 , 435/69.1 , 514/12 , 530/387.1 , 536/23.2 , 800/295

APPL-NO: 09/ 258031

DATE FILED: February 25, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09258031 A1 19990225

parent continuation-of PCT/EP97/04923 19970904 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	96 202 466.7	1996EP-96 202 466.7	September 4, 1996
EP	97 200 831.2	1997EP-97 200 831.2	March 19, 1997

PGPUB-DOCUMENT-NUMBER: 20020166141

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020166141 A1

TITLE: Antimicrobial peptides and methods of use

PUBLICATION-DATE: November 7, 2002

US-CL-CURRENT: 800/278, 435/320.1 , 435/410 , 536/23.2

APPL-NO: 09/ 950933

DATE FILED: September 11, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60232569 20000913 US

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application No. 60/232,569 filed Sep. 13, 2000, all of which is herein incorporated by reference.

PGPUB-DOCUMENT-NUMBER: 20020086008

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020086008 A1

TITLE: Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases

PUBLICATION-DATE: July 4, 2002

US-CL-CURRENT: 424/94.61, 435/200 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

APPL-NO: 09/ 977827

DATE FILED: October 15, 2001

RELATED-US-APPL-DATA:

child 09977827 A1 20011015

parent continuation-of 09343623 19990630 US GRANTED

parent-patent 6303118 US

PGPUB-DOCUMENT-NUMBER: 20020076402

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020076402 A1

TITLE: Enzyme-based fungicidal composition

PUBLICATION-DATE: June 20, 2002

US-CL-CURRENT: 424/94.61, 424/405

APPL-NO: 09/ 843169

DATE FILED: April 26, 2001

RELATED-US-APPL-DATA:

child 09843169 A1 20010426

parent continuation-of PCT/FR99/02645 19991028 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
FR	9813530	1998FR-9813530	October 28, 1998

PGPUB-DOCUMENT-NUMBER: 20020031504

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020031504 A1

TITLE: ANTIFUNGAL COMPOSITIONS

PUBLICATION-DATE: March 14, 2002

US-CL-CURRENT: 424/94.1, 424/405

APPL-NO: 09/ 202056

DATE FILED: December 7, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	96201651.5	1996EP-96201651.5	June 7, 1996

PCT-DATA:
APPL-NO: PCT/EP97/03046
DATE-FILED: Jun 9, 1997
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

PGPUB-DOCUMENT-NUMBER: 20010020300

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010020300 A1

TITLE: TRANSGENIC PATHOGEN-RESISTANT ORGANISM

PUBLICATION-DATE: September 6, 2001

US-CL-CURRENT: 800/279

APPL-NO: 09/ 138873

DATE FILED: August 24, 1998

CONTINUED PROSECUTION APPLICATION: CPA

RELATED-US-APPL-DATA:

child 09138873 A1 19980824

parent division-of 08812025 19970306 US GRANTED

parent-patent 5804184 US

child 08812025 19970306 US

parent division-of 08457797 19950601 US GRANTED

parent-patent 5689045 US

child 08457797 19950601 US

parent continuation-of 08134416 19931008 US ABANDONED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	P 42 34 131.0	1992DE-P 42 34 131.0	October 9, 1992

US-PAT-NO: 6521435

DOCUMENT-IDENTIFIER: US 6521435 B1

TITLE: Nucleic acid sequences encoding cell wall-degrading enzymes and use to engineer resistance to Fusarium and other pathogens

DATE-ISSUED: February 18, 2003

US-CL-CURRENT: 435/206, 435/183 , 435/200 , 435/252.3 , 435/320.1 , 435/419 , 435/468 , 435/69.1 , 536/23.2 , 800/295 , 800/298 , 800/320.3

APPL-NO: 09/ 649747

DATE FILED: August 28, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications Nos. 60/224,946, filed Aug. 11, 2000 and 60/151,582, filed Aug. 30, 1999. The disclosure of each of said provisional application is incorporated herein by reference in its entirety.

US-PAT-NO: 6495737

DOCUMENT-IDENTIFIER: US 6495737 B1

TITLE: Methods and compositions for improving salicylic acid-independent systemic acquired disease resistance in plants

DATE-ISSUED: December 17, 2002

US-CL-CURRENT: 800/279, 435/69.1 , 536/23.6 , 536/24.1 , 800/278

APPL-NO: 08/ 909125

DATE FILED: August 11, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional application Ser. No. 60/024,033, filed Aug. 12, 1996.

US-PAT-NO: 6419922

DOCUMENT-IDENTIFIER: US 6419922 B1

TITLE: Candida saitoana compositions for biocontrol of plant postharvest decay

DATE-ISSUED: July 16, 2002

US-CL-CURRENT: 424/93.51, 424/94.6

APPL-NO: 09/ 324525

DATE FILED: June 2, 1999

PARENT-CASE:

This application claims priority from U.S. Provisional Application Ser. No. 60/088,300 which was filed on Jun. 5, 1998, now abandoned.

US-PAT-NO: 6310091

DOCUMENT-IDENTIFIER: US 6310091 B1

TITLE: Fungicidal saponin, CAY-1, and isolation thereof from Capsium species fruit

DATE-ISSUED: October 30, 2001

US-CL-CURRENT: 514/462, 514/451 , 514/452 , 514/468 , 549/343 , 549/344

APPL-NO: 09/ 661757

DATE FILED: September 14, 2000

US-PAT-NO: 6303118

DOCUMENT-IDENTIFIER: US 6303118 B1
See image for Certificate of Correction

TITLE: Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases

DATE-ISSUED: October 16, 2001

US-CL-CURRENT: 424/94.61, 435/209 , 536/23.2

APPL-NO: 09/ 343623

DATE FILED: June 30, 1999

PARENT-CASE:

This application is a divisional application of U.S. Ser. No. 08/486,839 filed on Jun. 7, 1995, issued as U.S. Pat. No. 5,928,928.

US-PAT-NO: 6291647

DOCUMENT-IDENTIFIER: US 6291647 B1
See image for Certificate of Correction

TITLE: Antifungal proteins, DNA coding therefor, and hosts incorporating same

DATE-ISSUED: September 18, 2001

US-CL-CURRENT: 530/370, 435/418 , 435/419 , 530/300 , 530/350

APPL-NO: 08/ 687580

DATE FILED: November 20, 1996

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
NL	94200321	February 9, 1994

PCT-DATA:
APPL-NO: PCT/EP95/00488
DATE-FILED: February 9, 1995
PUB-NO: WO95/21929
PUB-DATE: Aug 17, 1995
371-DATE: Nov 20, 1996
102(E)-DATE:Nov 20, 1996

US-PAT-NO: 6271438

DOCUMENT-IDENTIFIER: US 6271438 B1
See image for Certificate of Correction

TITLE: Transgenic pathogen-resistant plant

DATE-ISSUED: August 7, 2001

US-CL-CURRENT: 800/279, 424/94.2 , 424/94.61 , 435/200 , 435/209 , 435/320.1 , 435/69.1 , 514/12 , 536/23.2 , 800/301

APPL-NO: 09/ 138873

DATE FILED: August 24, 1998

PARENT-CASE:

This application is a divisional of prior application No. 08/812,025 filed Mar. 6, 1997, now U.S. Pat. No. 5,804,184, which, in turn, is a divisional of prior application No. 08/457,797, filed Jun. 1, 1995, now U.S. Pat. No. 5,689,045, which is a continuation of prior application No. 08/134,416, filed Oct. 6, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 34 131	October 9, 1992

US-PAT-NO: 6221406

DOCUMENT-IDENTIFIER: US 6221406 B1
See image for Certificate of Correction

TITLE: Enzyme pre-granules for granular fodder

DATE-ISSUED: April 24, 2001

US-CL-CURRENT: 426/63, 426/453 , 426/454 , 426/461 , 426/463

APPL-NO: 09/ 180617

DATE FILED: February 25, 1999

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	196 19 219	May 13, 1996

PCT-DATA:
APPL-NO: PCT/EP97/02306
DATE-FILED: May 6, 1997
PUB-NO: WO97/42837
PUB-DATE: Nov 20, 1997
371-DATE: Feb 25, 1999
102(E)-DATE:Feb 25, 1999

US-PAT-NO: 6087560

DOCUMENT-IDENTIFIER: US 6087560 A

TITLE: Transgenic fungal resistant plants expressing chitinase and glucanase, process for obtaining, and recombinant polynucleotides for uses therein

DATE-ISSUED: July 11, 2000

US-CL-CURRENT: 800/301, 435/252.3 , 435/320.1 , 800/279 , 800/305 , 800/306 , 800/309 , 800/312 , 800/313 , 800/315 , 800/316 , 800/317 , 800/317.1 , 800/317.2 , 800/317.3 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3 , 800/321

APPL-NO: 08/ 801563

DATE FILED: February 18, 1997

PARENT-CASE:

This application is a continuation of U.S. Ser. No. 08/047,413 filed Apr. 19, 1993, U.S. Pat. No. 5,670,706, which is a continuation-in-part of U.S. Ser. No. 07/647,831 filed Jan. 29, 1991, abandoned, the disclosures of which are hereby incorporated herein by reference.

US-PAT-NO: 6066491

DOCUMENT-IDENTIFIER: US 6066491 A

TITLE: Process for obtaining fungal resistant plants with recombinant polynucleotides encoding .beta.-1,3-glucanase modified for apoplast targeting

DATE-ISSUED: May 23, 2000

US-CL-CURRENT: 435/252.3, 435/252.2 , 435/320.1

APPL-NO: 08/ 229050

DATE FILED: April 18, 1994

PARENT-CASE:

This application is a continuation of application Ser. No. 07/647,831, filed Jan. 29, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
NL	9000222	January 30, 1990

US-PAT-NO: 6057142

DOCUMENT-IDENTIFIER: US 6057142 A
See image for Certificate of Correction

TITLE: Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases

DATE-ISSUED: May 2, 2000

US-CL-CURRENT: 435/209, 435/252.3 , 435/320.1 , 435/325 , 536/23.2

APPL-NO: 09/ 151011

DATE FILED: September 10, 1998

PARENT-CASE:

This is a division application of application Ser. No. 08/486,839 filed Jun. 17, 1995 now U.S. Pat. No. 5,928,928.

US-PAT-NO: 5994625

DOCUMENT-IDENTIFIER: US 5994625 A

TITLE: Antifungal chitin binding proteins and DNA coding therefor

DATE-ISSUED: November 30, 1999

US-CL-CURRENT: 800/279, 435/200 , 435/209 , 435/252.2 , 435/320.1 , 435/418 , 435/419 , 435/421 , 435/468 , 435/469 , 435/69.1 , 536/23.6 , 800/265 , 800/268 , 800/294 , 800/298 , 800/301

APPL-NO: 08/ 935886

DATE FILED: September 23, 1997

PARENT-CASE:

This application is a continuation of application(s) Ser. No. 08/411,640 filed on Apr. 5, 1995, now abandoned, which is International Application PCT/EP93/02790 filed on Oct. 5, 1993 and which designated the U.S.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	92203071	October 5, 1992
EP	93201370	May 13, 1993

US-PAT-NO: 5993808

DOCUMENT-IDENTIFIER: US 5993808 A

TITLE: Chitinase, DNA coding therefor and plants containing same

DATE-ISSUED: November 30, 1999

US-CL-CURRENT: 424/94.61, 435/200 , 435/209

APPL-NO: 08/ 591629

DATE FILED: February 15, 1996

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	93202425	August 17, 1993

PCT-DATA:
APPL-NO: PCT/EP94/02761
DATE-FILED: August 17, 1994
PUB-NO: WO95/05467
PUB-DATE: Feb 23, 1995
371-DATE: Feb 15, 1996
102(E)-DATE:Feb 15, 1996

US-PAT-NO: 5981844

DOCUMENT-IDENTIFIER: US 5981844 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: November 9, 1999

US-CL-CURRENT: 800/301, 435/320.1 , 435/419 , 800/279

APPL-NO: 08/ 994418

DATE FILED: December 19, 1997

PARENT-CASE:

This Application is a Continuation of U.S. application Ser. No. 08/456,430 filed Jun. 1, 1995, now U.S. Pat. No. 5,703,044, which is a division of Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a Continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a Continuation in-Part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned. Such applications are herein incorporated by reference.

US-PAT-NO: 5928928

DOCUMENT-IDENTIFIER: US 5928928 A

TITLE: Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases

DATE-ISSUED: July 27, 1999

US-CL-CURRENT: 435/201, 435/183 , 530/350 , 536/23.1 , 536/24.3

APPL-NO: 08/ 486839

DATE FILED: June 7, 1995

US-PAT-NO: 5874626

DOCUMENT-IDENTIFIER: US 5874626 A
See image for Certificate of Correction

TITLE: Osmotin gene promoter and use thereof

DATE-ISSUED: February 23, 1999

US-CL-CURRENT: 800/279, 435/252.3 , 435/419 , 435/468 , 536/24.1 , 800/278 , 800/287 , 800/301 , 800/317.3

APPL-NO: 08/ 180428

DATE FILED: January 12, 1994

PARENT-CASE:

REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of U.S. patent application Ser. No. 08/065,147, filed May 20, 1993, now abandoned.

US-PAT-NO: 5804184

DOCUMENT-IDENTIFIER: US 5804184 A

TITLE: Transgenic pathogen-resistant organism

DATE-ISSUED: September 8, 1998

US-CL-CURRENT: 424/94.61, 424/94.2 , 435/200 , 435/209 , 514/12

APPL-NO: 08/ 812025

DATE FILED: March 6, 1997

PARENT-CASE:

This is a divisional of application No. 08/457,797, filed on Jun. 1, 1995, now U.S. Pat. No. 5,689,045, which is a continuation of Ser. No. 08/134,416, filed on Oct. 8, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 34 131.0	October 9, 1992

US-PAT-NO: 5801028

DOCUMENT-IDENTIFIER: US 5801028 A
See image for Certificate of Correction

TITLE: Osmotin gene promoter and use thereof

DATE-ISSUED: September 1, 1998

US-CL-CURRENT: 800/279, 435/200 , 435/320.1 , 435/419 , 536/23.6 , 536/24.5

APPL-NO: 08/ 482037

DATE FILED: June 7, 1995

PARENT-CASE:

REFERENCE TO RELATED APPLICATION

This application is a division of application Ser. No. 08/180,428, filed Jan. 12, 1994, abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 08/065,147, filed May 20, 1993, now pending.

US-PAT-NO: 5703044

DOCUMENT-IDENTIFIER: US 5703044 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: December 30, 1997

US-CL-CURRENT: 514/12, 514/2 , 514/8 , 530/372 , 530/376

APPL-NO: 08/ 456430

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional of application Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-PAT-NO: 5702605

DOCUMENT-IDENTIFIER: US 5702605 A

TITLE: Slime hydrolase producing bacterium and process for producing slime hydrolase

DATE-ISSUED: December 30, 1997

US-CL-CURRENT: 210/632, 162/161 , 210/764 , 422/28 , 435/183 , 435/252.1 , 435/264 , 435/267 , 435/822

APPL-NO: 08/ 776396

DATE FILED: January 28, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-200083	August 1, 1994

PCT-DATA:
APPL-NO: PCT/JP95/01513
DATE-FILED: July 31, 1995
PUB-NO: WO96/04370
PUB-DATE: Feb 15, 1996
371-DATE: Jan 28, 1997
102(E)-DATE:Jan 28, 1997

US-PAT-NO: 5695939

DOCUMENT-IDENTIFIER: US 5695939 A

TITLE: Plant defense genes and plant defense regulatory elements

DATE-ISSUED: December 9, 1997

US-CL-CURRENT: 435/6, 536/23.2 , 536/24.3

APPL-NO: 08/ 379259

DATE FILED: January 27, 1995

PARENT-CASE:

This application is a divisional application of U.S. Ser. No. 07/704,288, filed May 22, 1991, now U.S. Pat. No. 5,399,680, the entire contents of which is hereby incorporated by reference herein.

US-PAT-NO: 5689045

DOCUMENT-IDENTIFIER: US 5689045 A
See image for Certificate of Correction

TITLE: Transgenic pathogen-resistant plant

DATE-ISSUED: November 18, 1997

US-CL-CURRENT: 800/265, 435/200 , 435/209 , 435/320.1 , 435/69.1 , 435/70.1 , 47/DIG.1 , 536/23.2 , 536/23.6 , 536/23.7 , 800/279 , 800/301

APPL-NO: 08/ 457797

DATE FILED: June 1, 1995

PARENT-CASE:

This application is a continuation of application Ser. No. 08/134,416, filed on Oct. 8, 1993, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 34 131.0	October 9, 1992

US-PAT-NO: 5677175

DOCUMENT-IDENTIFIER: US 5677175 A

TITLE: Plant pathogen induced proteins

DATE-ISSUED: October 14, 1997

US-CL-CURRENT: 800/301, 435/320.1 , 435/410 , 435/419 , 435/69.1

APPL-NO: 08/ 728956

DATE FILED: October 11, 1996

PARENT-CASE:

This is a continuation-in-part application of U.S. application Ser. No. 60/005,362, filed Oct. 13, 1995, abandoned.

US-PAT-NO: 5670706

DOCUMENT-IDENTIFIER: US 5670706 A

TITLE: Fungal resistant plants, process for obtaining fungal resistant plants and recombinant polynucleotides for use therein

DATE-ISSUED: September 23, 1997

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 800/294 , 800/301 , 800/317.4

APPL-NO: 08/ 047413

DATE FILED: April 19, 1993

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 07/647,831 filed 29 Jan. 1991, now abandoned.

US-PAT-NO: 5608151

DOCUMENT-IDENTIFIER: US 5608151 A

TITLE: Anti-microbial proteins

DATE-ISSUED: March 4, 1997

US-CL-CURRENT: 800/298, 435/252.3 , 435/69.1 , 536/23.6 , 800/301 , 800/320.1

APPL-NO: 08/ 420526

DATE FILED: April 12, 1995

PARENT-CASE:

This is a divisional of application Ser. No. 08/209,923, filed on Feb. 22, 1994, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9303725	February 24, 1993

US-PAT-NO: 5607919

DOCUMENT-IDENTIFIER: US 5607919 A

TITLE: Anti-microbial proteins

DATE-ISSUED: March 4, 1997

US-CL-CURRENT: 514/12, 530/370

APPL-NO: 08/ 543238

DATE FILED: October 13, 1995

PARENT-CASE:

This is a continuation of application Ser. No. 08/209,923, filed on Feb. 22, 1994, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9303725	February 24, 1993

US-PAT-NO: 5559034

DOCUMENT-IDENTIFIER: US 5559034 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: September 24, 1996

US-CL-CURRENT: 435/320.1, 435/252.3 , 435/69.1 , 514/12 , 514/2 , 514/8 , 530/372 , 530/376 , 536/22.1 , 536/23.1 , 536/23.6

APPL-NO: 08/ 457552

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional application of Ser. No. 08/178,708, filed Jan. 10, 1994, which is a continuation-in-part of Ser. No. 07,505,781, filed Apr. 6, 1990, now abandoned which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-PAT-NO: 5521153

DOCUMENT-IDENTIFIER: US 5521153 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: May 28, 1996

US-CL-CURRENT: 514/2, 514/12 , 514/8 , 530/372 , 530/376

APPL-NO: 08/ 178708

DATE FILED: January 10, 1994

PARENT-CASE:

This Application is a continuation-in-part application of U.S. application Ser. No. 07/505,781, filed Apr. 6, 1990, which is a continuation-in-part Application of U.S. application Ser. No. 07/104,755 filed Oct. 2, 1987, both now abandoned. Such applications are herein incorporated by reference .

US-PAT-NO: 5399680

DOCUMENT-IDENTIFIER: US 5399680 A

TITLE: Rice chitinase promoter

DATE-ISSUED: March 21, 1995

US-CL-CURRENT: 536/24.1, 435/418 , 435/419 , 435/69.1 , 435/91.3

APPL-NO: 07/ 704288

DATE FILED: May 22, 1991

US-PAT-NO: 4032663

DOCUMENT-IDENTIFIER: US 4032663 A

TITLE: Process for using cell wall-lysing enzymes

DATE-ISSUED: June 28, 1977

US-CL-CURRENT: 426/51, 426/61 , 435/200 , 435/201 , 435/203 , 435/209 , 435/223 , 435/839 , 435/853 , 435/911 , 435/917 , 435/921 , 435/922 , 435/923 , 435/933 , 435/942

APPL-NO: 05/ 675448

DATE FILED: April 9, 1976

PARENT-CASE:

This is a division of application Ser. No. 522,304, filed Nov. 8, 1974, now U.S. Pat. No. 3,969,189, issued July 13, 1976, which in turn is a continuation of application Ser. No. 314,933 filed Dec. 14, 1972, now U.S. Pat. No. 3,890,198.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JA	46-100700	December 14, 1971
JA	47-9486	January 27, 1972

US-PAT-NO: 3969189

DOCUMENT-IDENTIFIER: US 3969189 A

TITLE: Cell wall-lysing complex enzymes and a process for the production thereof

DATE-ISSUED: July 13, 1976

US-CL-CURRENT: 435/206, 426/51 , 435/200 , 435/209 , 435/267 , 435/911

DISCLAIMER DATE: 19920617

APPL-NO: 05/ 522304

DATE FILED: November 8, 1974

PARENT-CASE:

This is a continuation of application Ser. No. 314,933, filed Dec. 14, 1972, now U.S. Pat. No. 3,890,198.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JA	46-100700	December 14, 1971
JA	47-9486	January 27, 1972

US-PAT-NO: 3890198

DOCUMENT-IDENTIFIER: US 3890198 A

TITLE: Cell wall-lysing complex enzymes and a process for the production thereof

DATE-ISSUED: June 17, 1975

US-CL-CURRENT: 435/206, 435/259 , 435/814 , 435/816 , 435/911

APPL-NO: 05/ 314933

DATE FILED: December 14, 1972

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JA	46-100700	December 14, 1971
JA	47-9486	January 27, 1972

US-PAT-NO: 6087560

DOCUMENT-IDENTIFIER: US 6087560 A

TITLE: Transgenic fungal resistant plants expressing chitinase and glucanase, process for obtaining, and recombinant polynucleotides for uses therein

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cornelissen; Bernardus J. C.	Warmond		N/A	NL
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL
Meulenhoff; Elisabeth J. S.	Amsterdam		N/A	NL
van Roekel; Jeroen S. C.	Amsterdam		N/A	NL
Sela-Buurlage; Marianne	Amersfoort		N/A	NL
Beatrix	Leiden	N/A	N/A	NL
Vloemans; Alexandra Aleida	Lafayette	IN	N/A	N/A
Woloshuk; Charles Peter	Oegstgeest		N/A	NL
Bol; John Ferdinand	Leiden	N/A	N/A	NL
Linthorst; Hubertus J. M.				

APPL-NO: 08/ 801563

DATE FILED: February 18, 1997

PARENT-CASE:

This application is a continuation of U.S. Ser. No. 08/047,413 filed Apr. 19, 1993, U.S. Pat. No. 5,670,706, which is a continuation-in-part of U.S. Ser. No. 07/647,831 filed Jan. 29, 1991, abandoned, the disclosures of which are hereby incorporated herein by reference.

US-CL-CURRENT: 800/301, 435/252.3 , 435/320.1 , 800/279 , 800/305 , 800/306 , 800/309 , 800/312 , 800/313 , 800/315 , 800/316 , 800/317 , 800/317.1 , 800/317.2 , 800/317.3 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3 , 800/321

ABSTRACT:

Plants are provided with improved resistance against pathogenic fungi. They are genetically transformed with one or more polynucleotides which essentially comprise one or more genes encoding plant chitinases and .beta.-1,3-glucanases. Preferred are the intracellular forms of the said hydrolytic enzymes, especially preferred are those forms which are targeted to the apoplastic space of the plant by virtue of the modification of the genes encoding the said enzymes. Particularly preferred are plants exhibiting a relative overexpression of at least one gene encoding a chitinase and one gene encoding a .beta.-1,3-glucanase.

25 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Brief Summary Text - BSTX (19):

More recently a purified endo-.beta.-1,3-**glucanase** from tomato in combination with an exo-.beta.-1,3-**glucanase** of fungal origin were shown to be capable of hydrolysing isolated cell walls of the fungus Verticillium albo-atrum. Each of the preparations separately did not have activity (Young & Pegg, 1982). A purified .beta.-1,3-**glucanase** from soybean (Keen & Yoshikawa, 1983), as well as a purified **chitinase** from bean (Boller et al., 1983) have also been shown to be capable of degrading isolated cell walls of fungi in vitro. When pea **chitinase** and .beta.-1,3-**glucanase** were tested on isolated cell walls of Fusarium solani, both appeared to be active; in combination they appeared to work **synergistically** (Mauch et al., 1988b).

Detailed Description Text - DETX (127):

Synergistic Effect of **Glucanase** on Antifungal Activity of **Chitinase**

Detailed Description Text - DETX (128):

Samsun NN tobacco plants were transformed with pMOG512 to constitutively express the modified intracellular **glucanase** gene(line 1); with pMOG512+pMOG289 to constitutively express the modified intracellular **chitinase** gene and the modified intracellular **glucanase** gene (line 2) and with pMOG189 to express the modified intracellular **chitinase** gene (line 3; see example 10). The plant lines were selected for high levels of expression of each chimeric gene. From each of the selected lines extracellular fluid (EF) (Parent & Asselin, 1984) and total leaf protein extracts (TE) (Kaufmann et al., 1987) were prepared. Initial dilutions were made of EF and TE of lines 2 and 3 to contain a **chitinase** activity of approximately 2000 cpm (see example 2). The initial dilutions of EF and TE of line 1 were equal to those of line 3. Subsequently, dilution series were made of the initial dilutions and these were tested for antifungal activity. No difference was found in antifungal activity between dilution series of EF and of TE. Moreover the highest antifungal activity was found in the (diluted) extracts of line 2. Apparently, the apoplast-targeted intracellular **glucanase has a synergistic** effect on the antifungal activity of the apoplast-targeted intracellular **chitinase**.

Claims Text - CLTX (11):

11. A plant transformed with a first expression system encoding a plant extracellular **chitinase** and a second expression system encoding a plant .beta.-1,3-**glucanase,** wherein expression of said **chitinase** and said .beta.-1,3-**glucanase** in said plant results in a **synergistic** antifungal effect.

Claims Text - CLTX (12):

12. A plant transformed with a first expression system encoding a plant intracellular **chitinase** and a second expression system encoding a plant extracellular .beta.-1,3-**glucanase,** wherein expression of said **chitinase** and said .beta.-1,3-**glucanase** in said plant results in a **synergistic** antifungal effect.

Claims Text - CLTX (17):

17. A recombinant polynucleotide for overexpression of a first gene encoding a plant extracellular **chitinase** and a second gene encoding a plant .beta.-1,3-**glucanase,** wherein said plant extracellular **chitinase** and said plant .beta.-1,3-**glucanase,** when produced in combination, exhibit a **synergistic** antifungal effect in said plant, which polynucleotide comprises in operable linkage:

US-PAT-NO: 6066491

DOCUMENT-IDENTIFIER: US 6066491 A

TITLE: Process for obtaining fungal resistant plants with recombinant polynucleotides encoding .beta.-1,3-glucanase modified for apoplast targeting

DATE-ISSUED: May 23, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cornelissen; Bernardus Johannes Clemens	Warmond	N/A	N/A	NL
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL

APPL-NO: 08/ 229050

DATE FILED: April 18, 1994

PARENT-CASE:

This application is a continuation of application Ser. No. 07/647,831, filed Jan. 29, 1991, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
NL	9000222	January 30, 1990

US-CL-CURRENT: 435/252.3, 435/252.2 , 435/320.1

ABSTRACT:

Plants are provided with improved resistance against pathogenic fungi. They are genetically transformed with one or more polynucleotides which essentially comprise one or more genes encoding plant and .beta.-1,3-glucanases. Preferred are the intracellular forms of the said hydrolytic enzymes, especially preferred are those forms which are targeted to the apoplastic space of the plant by virtue of the modification of the genes encoding the said enzymes. Particularly preferred are plants exhibiting a relative overexpression of at least one gene encoding a .beta.-1,3-glucanase.

7 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Brief Summary Text - BSTX (18):

More recently a purified endo-.beta.-1,3-**glucanase** from tomato in combination with an exo-.beta.-1,3-**glucanase** of fungal origin were shown to be capable of hydrolysing isolated cell walls of the fungus Verticillium albo-atrum. Each of the preparations separately did not have activity (Young & Pegg, 1982). A purified .beta.-1,3-**glucanase** from soybean (Keen & Yoshikawa, 1983), as well as a purified **chitinase** from bean (Boller et al., 1983) have also been shown to be capable of degrading isolated cell walls of fungi in vitro. When pea **chitinase** and .beta.-1,3-**glucanase** were tested on isolated cell walls of Fusarium solani, both appeared to be active; in combination they appeared to work **synergistically** (Mauch et al., 1988b).

Detailed Description Text - DETX (123):

Synergistic Effect of **Glucanase** on Antifungal Activity of **Chitinase**

Detailed Description Text - DETX (124):

Samsun NN tobacco plants were transformed with pMOG512 to constitutively express the modified intracellular **glucanase** gene(line 1); with pMOG512+pMOG289 to constitutively express the modified intracellular **chitinase** gene and the modified intracellular **glucanase** gene (line 2) and with pMOG189 to express the modified intracellular **chitinase** gene (line 3; see example 10). The plant lines were selected for high levels of expression of each chimeric gene. From each of the selected lines extracellular fluid (EF) (Parent & Asselin, 1984) and total leaf protein extracts (TE) (Kaufmann et al., 1987) were prepared. Initial dilutions were made of EF and TE of lines 2 and 3 to contain a **chitinase** activity of approximately 2000 cpm (see example 2). The initial dilutions of EF and TE of line 1 were equal to those of line 3. Subsequently, dilution series were made of the initial dilutions and these were tested for antifungal activity. No difference was found in antifungal activity between dilution series of EF and of TE. Moreover the highest antifungal activity was found in the (diluted) extracts of line 2. Apparently, the apoplast-targeted intracellular **glucanase has a synergistic** effect on the antifungal activity of the apoplast-targeted intracellular **chitinase**.

US-PAT-NO: 5994625

DOCUMENT-IDENTIFIER: US 5994625 A

TITLE: Antifungal chitin binding proteins and DNA coding therefor

DATE-ISSUED: November 30, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL
Sela-Buurlage; Marianne	Amersfoort	N/A	N/A	NL
Beatrix	Leiden	N/A	N/A	NL
Bres-Vloemans; Alexandra	Leiden	N/A	N/A	NL
Aleida	Haarlem	N/A	N/A	NL
Ponstein; Anne Silene	Warmond	N/A	N/A	NL
Apotheker-De Groot; Marion				
Cornelissen; Bernardus Johannes Clemens				

APPL-NO: 08/ 935886

DATE FILED: September 23, 1997

PARENT-CASE:

This application is a continuation of application(s) Ser. No. 08/411,640 filed on Apr. 5, 1995, now abandoned, which is International Application PCT/EP93/02790 filed on Oct. 5, 1993 and which designated the U.S.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	92203071	October 5, 1992
EP	93201370	May 13, 1993

US-CL-CURRENT: 800/279, 435/200 , 435/209 , 435/252.2 , 435/320.1 , 435/418 , 435/419 , 435/421 , 435/468 , 435/469 , 435/69.1 , 536/23.6 , 800/265 , 800/268 , 800/294 , 800/298 , 800/301

ABSTRACT:

Chimeric genes encoding antifungal chitin binding proteins (antifungal CBPs) with very low chitinase activity (10% or less than that of the class-I chitinases from tobacco). Also substantially pure DNA sequences encoding antifungal CBP are provided for the obtention of transgenic plants producing antifungal CBP. Plants expressing an antifungal CBP gene, optionally in combination with a plant expressible glucanase gene, show reduced susceptibility to fungi.

31 Claims, 5 Drawing figures

Exemplary Claim Number: 1,24

Number of Drawing Sheets: 5

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Brief Summary Text - BSTX (12):

The present invention provides a new class of antifungal chitin binding proteins, which are characterized in that they have low **chitinase** activity, a molecular weight of at least 15 kDa, and a strong **synergistic** antifungal effect in combination with 1,3-.beta.-**glucanases**; the antifungal effect of these proteins is not markedly decreased by divalent cations. Preferred antifungal chitin binding proteins are those which have an estimated molecular weight of about 20 kDa using SDS-PAGE and are obtainable from tobacco. Except for the hevein domain, the CBPs according to the invention do not bear much resemblance to the class-I **chitinases**, as the latter not only have a dissimilar molecular weight, but also lack substantive amino acid homology with the CBPs according to the invention.

Detailed Description Text - DETX (3):

The new CBPs are characterized by a molecular weight in the range of 15 to 25 kDa of the mature protein, a a drastic **synergistic** antifungal effect in combination with .beta.-1,3-**glucanase** and low **chitinase** activity (not more than 10%, more particularly not more than 5% of the class-I **chitinases** from tobacco as determined with the tritiated chitin method according to Molano et al., 1977, supra). A composition containing 5 .mu.g/ml tobacco CBP and 0.5 .mu.g/ml intracellular .beta.-1,3-**glucanase** from tobacco almost completely inhibited the growth of Fusarium solani and Trichoderma viride.

Detailed Description Text - DETX (34):

In European Pat. No. Application 440 304 A1 it was disclosed that simultaneous overexpression of a plant expressible **glucanase** gene in conjunction with an intracellular class-I **chitinase** from tobacco in transgenic plants results in a higher level of resistance to fungi than in plants expressing a plant expressible class-I **chitinase** alone. Since expression of **glucanase** alone does not yield resistant plants, it may be concluded that there is a **synergistic** effect of **glucanase** and intracellular class-I **chitinases**.

Detailed Description Text - DETX (35):

Both **chitinases, glucanases** and the new antifungal chitin binding proteins accumulate in infected plant tissues upon an incompatible pathogen-plant

interaction. Apparently, the **synergizing** effect of combinations of pathogen induced proteins is a more general phenomenon that has important consequences for the engineering of fungal resistant plants.

Detailed Description Text - DETX (36):

From these observations we predict, that the antifungal CBPs according to the invention will show a **synergistic** effect with many other proteins that bind to chitin or degrade chitin such as **chitinases**. Examples of **synergizing** proteins that may be used in combination with antifungal CBPs according to the invention include, but are not limited to, .beta.-1,3-**glucanases and chitinases** which are obtainable from barley (Swegle M. et al., 1989, Plant Mol. Biol. 12, 403-412; Balance G. M. et al., 1976, Can. J. Plant Sci. 56, 459-466 Hoj P. B. et al., 1988, FEBS Lett. 230, 67-71; Hoj P. B. et al., 1989, Plant Mol. Biol. 13, 31-42 1989), bean (Boller T. et al, 1983, Planta 157, 22-31; Broglie K. E. et al. 1986, Proc. Natl. Acad. Sci. USA 83, 6820-6824; Vdgeli U. et al., 1988 Planta 174, 364-372); Mauch F. & Staehelin L. A., 1989, Plant-Cell 1, 447-457); cucumber (Metraux J. P. & Boller T. (1986), Physiol. Mol. Plant Pathol. 28, 161-169); leek (Spanu P. et al., 1989, Planta 177, 447-455); maize (Nasser W. et al., 1988, Plant Mol. Biol. 11, 529-538), oat (Fink W. et al., 1988, Plant Physiol. 88, 270-275), pea (Mauch F. et al. 1984, Plant Physiol. 76, 607-611; Mauch F. et al., 1988, Plant Physiol. 87, 325-333), poplar (Parsons, T. J. et al, 1989, P.N.A.S. 86, 7895-7899), potato (Gaynor J. J. 1988, Nucl. Acids Res. 16, 5210; Kombrink E. et al. 1988, Proc. Natl. Acad. Sci. USA 85, 782-786; Laflamme D. and Roxby R., 1989, Plant Mol. Biol. 13, 249-250), tobacco (e.g. Legrand M. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 6750-6754; Shinshi H. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 89-93), tomato (Joosten M. H. A. & De Wit P. J. G. M. 1989, Plant Physiol. 89, 945-951), wheat (Molano J. et al., 1979, J. Biol. Chem. 254, 4901-4907), and the like.

Detailed Description Text - DETX (59):

The combination of 1 .mu.g CBP and 0.5 .mu.g **glucanase** causes lysis (70%) as well as a strong growth inhibiting effect on Fusarium solani. It is concluded that CBP has a **synergistic** antifungal effect in combination with **glucanases** as well as with class-I **chitinases**.

Detailed Description Text - DETX (60):

The results in table 2 indicate a **synergistic** antifungal effect of CBP and intracellular **glucanase** and at least an additive effect of CBP and class-I **chitinase** on Alternaria radicina. At 5 .mu.g per well, CBP has a growth inhibitory effect on Alternaria radicina, albeit rather weak.

Detailed Description Paragraph Table - DETL (1):

TABLE 1 _____ Antifungal effect of CBP

and **synergistic** antifungal effect of CBP with intracellular .beta.-1,3-**glucanase** or intracellular class-I **chitinase** on Fusarium solani glu-I -- 0.5 .mu.gr -- CBP chi-I -- -- 0.5 .mu.gr _____ 0 GI = 0 <5%, GI = 1 <5%, GI = 0 1 .mu.gr GI = 1 70%, GI = 3 <5%, GI = 3 5 .mu.gr GI = 3 70%, GI = 3 <5%, GI = 3 5 .mu.gr (den) GI = 0 <5%, GI = 0 <5%, GI = 0 _____ Lysis is indicated by a percentage with respect to untreated control. GI: growth inhibition; a scale of 0-4 is used, 0 = no visible inhibition, 1 weak inhibition, 2 = moderate inhibition, 3 = strong inhibition, 4 = very strong inhibition. (den) = denatured protein mixtures.

Claims Text - CLTX (1):

1. A chimeric gene comprising a polynucleotide sequence and a heterologous promoter, said polynucleotide sequence encoding an antifungal chitin binding protein that is obtainable from a plant, has a molecular weight in the range of 15 to 25 kDa, has a **synergistic** antifungal activity in combination with intracellular 1,3-.beta.-**glucanases,** a low **chitinase** activity, and reacts with antisera that recognize a chitin binding protein occurring naturally in tobacco, said polynucleotide sequence being under the control of the heterologous promoter, wherein said polynucleotide hybridizes under stringent conditions to the complement of a DNA sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 13.

Claims Text - CLTX (24):

21. A chimeric gene comprising a polynucleotide sequence and a heterologous promoter, wherein said heterologous promoter does not naturally promote expression of said polynucleotide sequence, and wherein the polynucleotide sequence encodes an antifungal chitin binding protein that is obtainable from tobacco, has a molecular weight of about 20 kDa, shows a **synergistic** antifungal activity in combination with intracellular 1,3-.beta.-**glucanase** from tobacco and has less than 5% of the **chitinase** activity of tobacco class-I **chitinase**.

Claims Text - CLTX (26):

23. A substantially pure polynucleotide sequence encoding an antifungal chitin binding protein from a tobacco plant, said antifungal chitin binding protein having a molecular weight in the range of 15 to 25 kDa, a **synergistic** antifungal activity in combination with intracellular 1,3-.beta.-**glucanases** and low **chitinase** activity, said antifungal chitin binding protein reacting with antisera that recognize a chitin binding protein occurring naturally in tobacco.

Claims Text - CLTX (28):

25. A method for reducing susceptibility of a plant or progeny of the plant

to fungi comprising (a) transforming the plant with a polynucleotide sequence that hybridizes under stringent conditions to the complement of a DNA sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 13 said polynucleotide sequence encoding an antifungal chitin binding protein that is obtainable from a plant, has a molecular weight in the range of 15 to 20 kDa, a **synergistic** antifungal activity in combination with intracellular 1,3-.beta.-**glucanases** and low **chitinase** activity, said antifungal chitin binding protein reacting with antisera that recognize a chitin binding protein occurring naturally in tobacco, and (b) selecting a transformant having a reduced susceptibility to the fungi.

US-PAT-NO: 5981844

DOCUMENT-IDENTIFIER: US 5981844 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 994418

DATE FILED: December 19, 1997

PARENT-CASE:

This Application is a Continuation of U.S. application Ser. No. 08/456,430 filed Jun. 1, 1995, now U.S. Pat. No. 5,703,044, which is a division of Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a Continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a Continuation in-Part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned. Such applications are herein incorporated by reference.

US-CL-CURRENT: 800/301, 435/320.1 , 435/419 , 800/279

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

5 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Brief Summary Text - BSTX (7):

Certain enzymes have also been reported to **synergize** the effect of antifungal agents. Lysozyme has been reported to **synergize** the activity of amphotericin B against Candida albicans and Coccidioides immitis (Collins and Pappagianis (1974) Sabouraudia 12:329-340). Natural mixtures of mycolytic enzymes of fungal origin, designated mycolases, were reported to have a **synergistic** effect on the activity of the antifungal drugs amphotericin B and nystatin (Davies and Pope (1978) Nature 273:235-6; Pope and Davies (1979) Postgraduate Med. J. 55:674-676). The in vitro MICs (minimum inhibitory concentrations) of these antifungal drugs were lowered about 5 to 10-fold in combinations with mycolase. In related in vivo experiments in a mouse model, fungal mycolase was reported to enhance the effectiveness of amphotericin B and nystatin against systemic infection of C. albicans. It was suggested that mycolase, which was suggested to be a mixture of carbohydrases, enhanced penetration of the antibiotic into fungal cells. Fungal mycolases, alone, were described as very effective at releasing protoplasts from Aspergillus fumigatus and C. albicans in vitro and were also reported to have some effect, alone, against systemic fungal infection in the mouse model system. In contrast, a prepared mixture of the carbohydrases **chitinase** (.beta.-1,4 N-acetyl-D-glucosaminidase) and laminarinase (.beta.-1,3(4)-**glucanase**), while reported to effect protoplast release from A. fumigatus and C. albicans, did not enhance the effectiveness of amphotericin B and nystatin in vivo. Recently, in similar in vitro and in vivo experiments with fungal mycolase/amphotericin B mixtures, only slight enhancement of antifungal activity by a fungal mycolase was reported (Chalkley et al. (1985) Sabouraudia 23:147-164). This report suggests that the difference in results compared to those reported earlier by Davies and Pope (supra) may be associated with the lower **chitinase** or lower .beta.-1,6-D-**glucanase** activities in their preparation of mycolase compared to that employed in the previous experiments. The specific enzymatic activities present in fungal mycolases have not been identified, and the specific protein or proteins in mycolase that may effect antibiotic enhancement have not been identified Some bacterial mycolases have also been reported to effect enhancements (about 2-fold) of the activity of amphotericin B (Oranusi and Trinci (1985) Microbios 43:17-30). Again, no specific enzyme activity was associated with **synergy**.

Brief Summary Text - BSTX (16):

Zeamatin has been isolated in substantially pure form by methods described herein, as demonstrated by the absence of contaminating protein bands in conventional protein gel electrophoresis, as shown in FIG. 4. The N-terminal amino acid sequence (30 amino acids) of zeamatin is provided in Table 5 (SEQ ID NO:1). Substantially pure zeamatin displays no detectable **chitinase** activity,

1,3 .beta.-glucanase, protease, ribonuclease, phospholipase C, mannanase, N-.beta.-acetylhexosaminidase or ribosome-inactivating protein activity as assessed by procedures described herein or well known in the art. Substantially pure zeamatin preparations include those in which the 22 kd protein represents about 90% or more of the total protein present in the preparation. In in vitro **synergy** plate assays, zeamatin was found to greatly enhance the anti-Candida activity of nikkomycin X or Z up to about 100 fold, while in liquid culture assays, enhancements of up to 1000 fold were observed. Greater enhancement of anti-Candida activity of nikkomycin X or Z is observed in agar-free medium containing low concentrations of peptone and peptides. Zeamatin also displayed significant enhancement (about 10-fold) of the activity of polyoxin against C. albicans and also enhanced (about 3-fold) the activity of amphotericin B against this yeast.

Drawing Description Text - DRTX (3):

FIGS. 2A-2B show elution profiles from CM-Sephadex.TM. column purification of zeamatin from corn protein extracts. A flow rate of 1 ml/min was employed in these separations. Protein in each 6 ml fraction was quantified by measurement of absorbance at 280 nm. Bound protein was eluted with a linear salt gradient (0.01 0.2 M NaCl). Four peaks were eluted. FIG. 2A displays the quantitative results of antifungal assays, while FIG. 2B displays the quantitative results of enzyme assays across the four peaks. Absorbance at 280 nm is represented in both A and B by closed circles, solid lines. The results of hyphal extension inhibition of T. reesei (open circles, solid line), hyphal extension inhibition of N. crassa (closed squares, dashed lines) and **synergistic** anti-Candida activity (closed triangles, dotted line) are presented on panel A. The results of **chitinase** (closed triangles, dotted line), **glucanase** (open squares, solid line) and .beta.-N-acetyl-hexosaminidase (closed circles, dashed line) assays are presented in panel B.

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and barley **AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn **AFP** preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they **synergized** with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, **synergized with all AFP** preparations, but **synergy** was particularly dramatic with corn-**AFP** preparations. Polyoxin **synergized** significantly with corn and wheat **AFP** preparations, while modest **synergy** was observed with combinations of amphotericin and **AFP** preparations from barley and corn. In contrast, no **synergy** was observed with papulocandin and **AFP** preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-**AFP** preparation (Table 1) when

chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using **synergy** with nikkomycin to inhibit the growth of C albicans as an activity assay, the **synergizing** activity in corn-**AFP** preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (5):

Since a significant loss in specific **synergizing** activity was observed in the conventional phenyl-Sepharose.TM. chromatography step, efforts were made to improve the purification of corn-SAFP activity. Improved purification of zeamatin was obtained by carrying out the CM-Sephadex.TM. chromatography at a slower flow rate than had been employed in previous separations and more importantly, by employing a novel phenyl-Sepharose.TM. chromatographic procedure. Slower elution in the CM-Sephadex.TM. step resulted in four distinct protein peaks (FIG. 2) rather than the three peaks observed previously (FIG. 1). **Synergistic** anti-Candida activity was found only in peak 3. Anti-Neurospora activity was also confined to peak 3, while anti-Trichoderma activity was observed in all peak fractions. All four peaks were also assayed for **chitinase, glucanase** and .beta.-N-acetylhexosaminidase activity. None of these enzyme activities coincided with the anti-Neurospora or **synergistic** anti-Candida activity of peak 3.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases** from Serratia marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in **glucanase** preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified **glucanase** preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both **chitinase and glucanase** called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

Detailed Description Text - DETX (16):

The mechanism by which SAFP **synergizes** the action of polyoxins, nikkomycins and amphotericins is not known. It was thought that SAFP might act to increase penetration of the antibiotics into the target fungi. This could occur as the result of degradation or permeabilization of the fungal cell wall by SAFP. Fungal cell walls are composed of chitin, glucans with .beta.-1,3 or

.beta.-1,6- linkages and mannans with .alpha.-1,6, .alpha.-1,2 or .alpha.-1,3-linkages. It has been demonstrated, however, that zeamatin, unlike other antimycotic agents, does not have **chitinase, glucanase** or mannanase activity. A more probable mechanism, supported by experiments described below, is that SAFP permeabilizes the fungal cell membrane. It is suggested that SAFP lyses fungi by direct insertion of the protein into fungal membranes to form transmembrane pores. Amphiphilic polypeptides may bind to cells through a cationic region of the molecule followed by insertion of a hydrophobic domain through the lipid bilayer of the membrane. For example, zeamatin's amphiphilic nature is suggested by the protein's late elution from CM-Sephadex.TM. (a cationic property) and its retarded passage through phenyl-Sepharose.TM. (a hydrophobic property). That zeamatin acts via cell membrane permeabilization is further supported by the rapid effect of low concentrations of SAFP's on fungi, even at 0.degree. C. For example, 1 g/ml zeamatin induces hyphal rupture in less than 15 seconds at 23.degree. C. This rapid rupture suggests a non-enzymatic mechanism of action. The operability and utility of the SAFPs of the present invention are, however, not dependent upon these suggested mechanisms, and the practice of the present invention does not require characterization of the specific activity of an SAFP. Similarities in structure (similar molecular weights, similar elution behavior on chromatography and homologies in N-terminal sequence) of zeamatin, sormatin and avematin, and more importantly, their common function in **synergism** of anti-fungal activity, indicate that these proteins and other protein strains displaying this function represent a class of proteins (SAFPs) which act by an analogous mechanism.

Detailed Description Text - DETX (34):

A second procedure was found to result in improved purification of zeamatin. Ammonium sulfate fractionation of corn protein extract was performed as described above. The dialyzed 30%-55% fraction was subjected to CM-Sephadex.TM. chromatography, essentially as described above. However, the chromatography was carried out at a slower flow rate (1 ml/min), which resulted in the elution of four distinct peaks (FIG. 2). **Synergistic** anti-Candida activity was confined to peak 3. This peak was also found to contain growth inhibitory activity against Neurospora crassa Anti-Neurospora activity was found only in corn **AFP** preparations, not in **AFP** preparations of wheat and barley. Anti-Trichoderma activity was found in all four peaks. **Chitinase, glucanase** (.beta.1,3- and .beta.1,6-) and .beta.-N-acetylhexosaminidase activities were also assayed across the four peaks. **Chitinase** was found in all four peaks. A single peak of **glucanase** activity at fraction 47 and a single peak of .beta.-N-acetyl hexosaminidase at fraction 40 were detected. Anti-Neurospora and **synergistic** anti-Candida activity peaked at fraction 44. These antifungal activities did not coincide with any of the enzyme activities tested.

Detailed Description Text - DETX (36):

The three peak fractions from phenyl-Sepharose.TM. chromatography were assayed for enzyme activities and antifungal activities as shown in Table 2.

Peak 1 contained most of the **chitinase** activity, a small amount of **glucanase** activity, and most of the anti-Trichoderma activity. No anti-Neurospora or **synergistic** anti-Candida activity was found in peak 1. Peak 3 contained most of the **glucanase** activity and no other detectable enzymatic or antifungal activity. Peak 2 contained all of the anti-Neurospora and **synergistic** anti-Candida activity and a smaller activity against Trichoderma. Peak 2 contained no detectable **chitinase** activity and a very small amount of **glucanase**.

Detailed Description Text - DETX (56):

Zeamatin partially purified by CM-Sephadex.TM. (fraction CMS) displayed both **chitinase** and .beta.-1, 3 **glucanase** activity in addition to antifungal activity against T. reesi and N. crassa, and **synergistic** activity in combination with antifungalantibiotics, especially nikkomycin, against Candida albicans. Phenyl-Sepharose.TM.-purified zeamatin displayed no **chitinase,** mannanase or .beta.-N-acetylhexosaminidase activity, and little or no **glucanase** activity. **Synergistic** antifungal activity is not associated with the presence of **chitinase or glucanase** activity.

US-PAT-NO: 5874626

DOCUMENT-IDENTIFIER: US 5874626 A
See image for Certificate of Correction

TITLE: Osmotin gene promoter and use thereof

DATE-ISSUED: February 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bressan; Ray	W. Lafayette	IN	N/A	N/A
Hasegawa; Paul M.	W. Lafayette	IN	N/A	N/A

APPL-NO: 08/ 180428

DATE FILED: January 12, 1994

PARENT-CASE:

REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of U.S. patent application Ser. No. 08/065,147, filed May 20, 1993, now abandoned.

US-CL-CURRENT: 800/279, 435/252.3 , 435/419 , 435/468 , 536/24.1 , 800/278 , 800/287 , 800/301 , 800/317.3

ABSTRACT:

Described are an isolated DNA fragment incorporating an osmotin gene promoter sequence, recombinant DNA incorporating a foreign structural gene under control of an osmotin gene promoter sequence, as well as methods and transformants involving the isolated DNA fragment and recombinant DNA. Also described are methods for the inhibition of fungal, insect, nematode, and viral pathogens in a plant using such recombinant DNA.

19 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Detailed Description Text - DETX (14):

Thus, the present invention encompasses methods of protecting plants from

fungal infections by transforming plants with vectors comprising the osmotin promoter and a foreign structural gene encoding **chitinase, glucanase** or ribosome inactivating protein. The present invention also contemplates the transformation of plants with a combination of such vectors to provide a **synergistic** effect.

US-PAT-NO: 5801028

DOCUMENT-IDENTIFIER: US 5801028 A
See image for Certificate of Correction

TITLE: Osmotin gene promoter and use thereof

DATE-ISSUED: September 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bressan; Ray	W. Lafayette	IN	N/A	N/A
Hasegawa; Paul M.	W. Lafayette	IN	N/A	N/A

APPL-NO: 08/ 482037

DATE FILED: June 7, 1995

PARENT-CASE:

REFERENCE TO RELATED APPLICATION

This application is a division of application Ser. No. 08/180,428, filed Jan. 12, 1994, abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 08/065,147, filed May 20, 1993, now pending.

US-CL-CURRENT: 800/279, 435/200 , 435/320.1 , 435/419 , 536/23.6 , 536/24.5

ABSTRACT:

Described are an isolated DNA fragment incorporating an osmotin gene promoter sequence, recombinant DNA incorporating a foreign structural gene under control of an osmotin gene promoter sequence, as well as methods and transformants involving the isolated DNA fragment and recombinant DNA. Also described are methods for the inhibition of fungal, insect, nematode, and viral pathogens in a plant using such recombinant DNA.

14 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Detailed Description Text - DETX (14):

Thus, the present invention encompasses methods of protecting plants from

fungal infections by transforming plants with vectors comprising the osmotin promoter and a foreign structural gene encoding **chitinase, glucanas** or ribosome inactivating protein. The present invention also contemplates the transformation of plants with a combination of such vectors to provide a **synergistic** effect.

US-PAT-NO: 5703044

DOCUMENT-IDENTIFIER: US 5703044 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: December 30, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 456430

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional of application Ser. No. 08/178,708, filed Jan. 10, 1994, now U.S. Pat. No. 5,521,153, which is a continuation-in-part of Ser. No. 07/505,781, filed Apr. 6, 1990, now abandoned, which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-CL-CURRENT: 514/12, 514/2 , 514/8 , 530/372 , 530/376

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

26 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Brief Summary Text - BSTX (7):

Certain enzymes have also been reported to **synergize** the effect of antifungal agents. Lysozyme has been reported to **synergize** the activity of amphotericin B against Candida albicans and Coccidioides immitis (Collins and Pappagianis (1974) Sabouraudia 12:329-340). Natural mixtures of mycolytic enzymes of fungal origin, designated mycolases, were reported to have a **synergistic** effect on the activity of the antifungal drugs amphotericin B and nystatin (Davies and Pope (1978) Nature 273:235-6; Pope and Davies (1979) Postgraduate Med. J. 55:674-676). The in vitro MICs (minimum inhibitory concentrations) of these antifungal drugs were lowered about 5 to 10-fold in combinations with mycolase. In related in vivo experiments in a mouse model, fungal mycolase was reported to enhance the effectiveness of amphotericin B and nystatin against systemic infection of C. albicans. It was suggested that mycolase, which was suggested to be a mixture of carbohydrases, enhanced penetration of the antibiotic into fungal cells. Fungal mycolases, alone, were described as very effective at releasing protoplasts from Aspergillus fumigatus and C. albicans in vitro and were also reported to have some effect, alone, against systemic fungal infection in the mouse model system. In contrast, a prepared mixture of the carbohydrases **chitinase** (.beta.-1,4 N-acetyl-D-glucosaminidase) and laminarinase (.beta.-1,3(4)**-glucanase**), while reported to effect protoplast release from A. fumigatus and C. albicans, did not enhance the effectiveness of amphotericin B and nystatin in vivo. Recently, in similar in vitro and in vivo experiments with fungal mycolase/amphotericin B mixtures, only slight enhancement of antifungal activity by a fungal mycolase was reported (Chalkley et al. (1985) Sabouraudia 23:147-164). This report suggests that the difference in results compared to those reported earlier by Davies and Pope (supra) may be associated with the lower **chitinase** or lower .beta.1,6-D**-glucanase** activities in their preparation of mycolase compared to that employed in the previous experiments. The specific enzymatic activities present in fungal mycolases have not been identified, and the specific protein or proteins in mycolase that may effect antibiotic enhancement have not been identified. Some bacterial mycolases have also been reported to effect enhancements (about 2-fold) of the activity of amphotericin B (Oranusi and Trinci (1985) Microbios 4-3:17-30). Again, no specific enzyme activity was associated with **synergy**.

Brief Summary Text - BSTX (16):

Zeamatin has been isolated in substantially pure form by methods described herein, as demonstrated by the absence of contaminating protein bands in conventional protein gel electrophoresis, as shown in FIG. 4. The N-terminal amino acid sequence (30 amino acids) of zeamatin is provided in Table 5(SEQ ID NO:1). Substantially pure zeamatin displays no detectable **chitinase** activity, 1-3 .beta.**-glucanase,** protease, ribonuclease, phospholipase C, mannanase,

N-.beta.-acetylhexosaminidase or ribosome-inactivating protein activity as assessed by procedures described herein or well known in the art. Substantially pure zeamatin preparations include those in which the 22 kd protein represents about 90% or more of the total protein present in the preparation. In in vitro **synergy** plate assays, zeamatin was found to greatly enhance the anti-Candida activity of nikkomycin X or Z up to about 100 fold, while in liquid culture assays, enhancements of up to 1000 fold were observed. Greater enhancement of anti-Candida activity of nikkomycin X or Z is observed in agar-free medium containing low concentrations of peptone and peptides. Zeamatin also displayed significant enhancement (about 10-fold) of the activity of polyoxin against C. albicans and also enhanced (about 3-fold) the activity of amphotericin B against this yeast.

Drawing Description Text - DRTX (3):

FIG. 2 shows elution profiles from CM-Sephadex.TM. column purification of zeamatin from corn protein extracts. A flow rate of 1 ml/min was employed in these separations. Protein in each 6 ml fraction was quantified by measurement of absorbance at 280 nm. Bound protein was eluted with a linear salt gradient (0.01 0.2M NaCl). Four peaks were eluted. FIG. 2A displays the quantitative results of antifungal assays, while FIG. 2B displays the quantitative results of enzyme assays across the four peaks. Absorbance at 280 nm is represented in both A and B by closed circles, solid lines. The results of hyphal extension inhibition of T. reesei (open circles, solid line), hyphal extension inhibition of N. crassa (closed squares, dashed lines) and **synergistic** anti-Candida activity (closed triangles, dotted line) are presented on panel A. The results of **chitinase** (closed triangles, dotted fine), **glucanase** (open squares, solid line) and .beta.-N-acetyl-hexosaminidase (closed circles, dashed line) assays are presented in panel B.

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and barley **AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn **AFP** preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they **synergized** with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, **synergized with all AFP** preparations, but **synergy** was particularly dramatic with corn-**AFP** preparations. Polyoxin **synergized** significantly with corn and wheat **AFP** preparations, while modest **synergy** was observed with combinations of amphotericin and **AFP** preparations from barley and corn. In contrast, no **synergy** was observed with papulocandin and **AFP** preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-**AFP** preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple

protein peaks (FIG. 1). Using **synergy** with nikkomycin to inhibit the growth of C. albicans as an activity assay, the **synergizing** activity in corn-**AFP** preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (5):

Since a significant loss in specific **synergizing** activity was observed in the conventional phenyl-Sepharose.TM. chromatography step, efforts were made to improve the purification of corn-SAFP activity. Improved purification of zeamatin was obtained by carrying out the CM-Sephadex.TM. chromatography at a slower flow rate than had been employed in previous separations and more importantly, by employing a novel phenyl-Sepharose.TM. chromatographic procedure. Slower elution in the CM-Sephadex.TM. step resulted in four distinct protein peaks (FIG. 2) rather than the three peaks observed previously (FIG. 1). **Synergistic** anti-Candida activity was found only in peak 3. Anti-Neurospora activity was also confined to peak 3, while anti-Trichoderma activity was observed in all peak fractions. All four peaks were also assayed for **chitinase, glucanase** and .beta.-N-acetylhexosaminidase activity. None of these enzyme activities coincided with the anti-Neurospora or **synergistic** anti-Candida activity of peak 3.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases** from Serratia marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in **glucanase** preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified **glucanase** preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both **chitinase and glucanase** called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

Detailed Description Text - DETX (16):

The mechanism by which SAFP **synergizes** the action of polyoxins, nikkomycins and amphotericins is not known. It was thought that SAFP might act to increase penetration of the antibiotics into the target fungi. This could occur as the result of degradation or permeabilization of the fungal cell wall by SAFP. Fungal cell walls are composed of chitin, glucans with .beta.-1,3 or .beta.-1,6-linkages and mannans with .alpha.-1,6, .alpha.-1,2 or

.alpha.-1,3-linkages. It has been demonstrated, however, that zeamatin, unlike other antimycotic agents, does not have **chitinase, glucanase** or mannanase activity. A more probable mechanism, supported by experiments described below, is that SAFP permeabilizes the fungal cell membrane. It is suggested that SAFP lyses fungi by direct insertion of the protein into fungal membranes to form transmembrane pores. Amphiphilic polypeptides may bind to cells through a cationic region of the molecule followed by insertion of a hydrophobic domain through the lipid bilayer of the membrane. For example, zeamatin's amphiphilic nature is suggested by the protein's late elution from CM-Sephadex.TM. (a cationic property) and its retarded passage through phenyl-Sepharose.TM. (a hydrophobic property). That zeamatin acts via cell membrane permeabilization is further supported by the rapid effect of low concentrations of SAFP's on fungi, even at 0.degree. C. For example, 1 g/ml zeamatin induces hyphal rupture in less than 15 seconds at 23.degree. C. This rapid rapture suggests a non-enzymatic mechanism of action. The operability and utility of the SAFPs of the present invention are, however, not dependent upon these suggested mechanisms, and the practice of the present invention does not require characterization of the specific activity of an SAFP. Similarities in structure (similar molecular weights, similar elution behavior on chromatography and homologies in N-terminal sequence) of zeamatin, sormatin and avematin, and more importantly, their common function in **synergism** of anti-fungal activity, indicate that these proteins and other protein strains displaying this function represent a class of proteins (SAFPs) which act by an analogous mechanism.

Detailed Description Text - DETX (34):

A second procedure was found to result in improved purification of zeamatin. Ammonium sulfate fractionation of corn protein extract was performed as described above. The dialyzed 30%-55% fraction was subjected to CM-Sephadex.TM. chromatography, essentially as described above. However, the chromatography was carried out at a slower flow rate (1 ml/min), which resulted in the elution of four distinct peaks (FIG. 2). **Synergistic** anti-Candida activity was confined to peak 3. This peak was also found to contain growth inhibitory activity against Neurospora crassa. Anti-Neurospora activity was found only in corn **AFP** preparations, not in **AFP** preparations of wheat and barley. Anti-Trichederma activity was found in all four peaks. **Chitinase, glucanase** (.beta.1,3- and .beta.1,6-) and .beta.-N-acetylhexosaminidase activities were also assayed across the four peaks. **Chitinase** was found in all four peaks. A single peak of **glucanase** activity at fraction 47 and a single peak of .beta.-N-acetyl hexosaminidase at fraction 40 were detected. Anti-Neurospora and **synergistic** anti-Candida activity peaked at fraction 44. These antifungal activities did not coincide with any of the enzyme activities tested.

Detailed Description Text - DETX (36):

The three peak fractions from phenyl-Sepharose.TM. chromatography were assayed for enzyme activities and antifungal activities as shown in Table 2. Peak 1 contained most of the **chitinase** activity, a small amount of **glucanase**

activity, and most of the anti-Trichoderma activity. No anti-Neurospora or **synergistic** anti-Candida activity was found in peak 1. Peak 3 contained most of the **glucanase** activity and no other detectable enzymatic or antifungal activity. Peak 2 contained all of the anti-Neurospora and **synergistic** anti-Candida activity and a smaller activity against Trichederma. Peak 2 contained no detectable **chitinase** activity and a very small amount of **glucanase**.

Detailed Description Text - DETX (56):

Zeamatin partially purified by CM-Sephadex.TM. (fraction CMS) displayed both **chitinase** and .beta.-1,3 **glucanase** activity in addition to antifungal activity against T. reesi and N. crassa, and **synergistic** activity in combination with antifungalantibiotics, especially nikkomycin, against Candida albicans. Phenyl-Sepharose.TM.-purified zeamatin displayed no **chitinase,** mannanase or .beta.-N-acetylhexosaminidase activity, and little or no **glucanase** activity. **Synergistic** antifungal activity is not associated with the presence of **chitinase or glucanase** activity.

US-PAT-NO: 5695939

DOCUMENT-IDENTIFIER: US 5695939 A

TITLE: Plant defense genes and plant defense regulatory elements

DATE-ISSUED: December 9, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zhu; Qun	San Diego	CA	N/A	N/A
Lamb; Christopher J.	San Diego	CA	N/A	N/A

APPL-NO: 08/ 379259

DATE FILED: January 27, 1995

PARENT-CASE:

This application is a divisional application of U.S. Ser. No. 07/704,288, filed May 22, 1991, now U.S. Pat. No. 5,399,680, the entire contents of which is hereby incorporated by reference herein.

US-CL-CURRENT: 435/6, 536/23.2 , 536/24.3

ABSTRACT:

Novel chitinase gene, and its associated regulatory region, from a monocotyledon plant is described.

7 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Brief Summary Text - BSTX (5):

Chitinase (EC 3.2.1.14) catalyzes the hydrolysis of the .beta.-1,4 linkages of the N-acetyl-D-glucosamine polymer chitin. Chitin does not occur in higher plants, but is present in the cell walls of many fungi. **Chitinase,** which exhibits complex developmental and hormonal regulation, has been found in many species of higher plants. In addition, **chitinase** activity is markedly increased by wounding, ethylene, or microbial elicitors. Furthermore, **chitinase** is involved in the hypersensitive resistance response to microbial

attack. Purified plant **chitinase** attacks and partially digests isolated cell walls of potentially pathogenic fungi. It is this latter enzyme activity, rather than chitin-binding lectin activity, that is responsible for the inhibition of fungal growth. **Chitinase** and .beta.-**glucanase** exhibit **synergistic** antifungal activity in vitro. A number of pathogenesis-related proteins (also referred to as "PR proteins") have been found to be **chitinases or glucanases**.

US-PAT-NO: 5670706

DOCUMENT-IDENTIFIER: US 5670706 A

TITLE: Fungal resistant plants, process for obtaining fungal resistant plants and recombinant polynucleotides for use therein

DATE-ISSUED: September 23, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cornelissen; Bernardus J. C.	Warmond		N/A	NL
Melchers; Leo Sjoerd	Leiden	N/A	N/A	NL
Meulenhoff; Elisabeth J. S.	Amsterdam		N/A	NL
van Roekel; Jeroen S. C.	Amsterdam		N/A	NL
Sela-Buurlage; Marianne	Amersfoort		N/A	NL
Beatrix	Leiden	N/A	N/A	NL
Vloemans; Alexandra Aleida	Lafayette	IN	N/A	N/A
Woloshuk; Charles Peter	Oegstgeest		N/A	NL
Bol; John Ferdinand	Leiden	N/A	N/A	NL
Linthorst; Hubertus J. M.				

APPL-NO: 08/ 047413

DATE FILED: April 19, 1993

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 07/647,831 filed 29 Jan. 1991, now abandoned.

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 800/294 , 800/301 , 800/317.4

ABSTRACT:

Plants are provided with improved resistance against pathogenic fungi. They are genetically transformed with one or more polynucleotides which essentially comprise one or more genes encoding plant chitinases and .beta.-1,3-glucanases. Preferred are the intracellular forms of the said hydrolytic enzymes, especially preferred are those forms which are targeted to the apoplastic space of the plant by virtue of the modification of the genes encoding the said enzymes. Particularly preferred are plants exhibiting a relative overexpression of at least one gene encoding a chitinase and one gene encoding a .beta.-1,3-glucanase.

30 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Brief Summary Text - BSTX (19):

More recently a purified endo-.beta.-1,3-**glucanase** from tomato in combination with an exo-.beta.-1,3-**glucanase** of fungal origin were shown to be capable of hydrolysing isolated cell walls of the fungus Verticillium albo-atrum. Each of the preparations separately did not have activity (Young & Pegg, 1982). A purified .beta.-1,3-**glucanase** from soybean (Keen & Yoshikawa, 1983), as well as a purified **chitinase** from bean (Boller et al., 1983) have also been shown to be capable of degrading isolated cell walls of fungi in vitro. When pea **chitinase** and .beta.-1,3-**glucanase** were tested on isolated cell walls of Fusarium solani, both appeared to be active; in combination they appeared to work **synergistically** (Mauch et al., 1988b).

Detailed Description Text - DETX (116):

Synergistic effect of **glucanase** on antifungal activity of **chitinase**

Detailed Description Text - DETX (117):

Samsun NN tobacco plants were transformed with pMOG512 to constitutively express the modified intracellular **glucanase** gene (line 1); with pMOG512+pMOG289 to constitutively express the modified intracellular **chitinase** gene and the modified intracellular **glucanase** gene (line 2) and with pMOG189 to express the modified intracellular **chitinase** gene (line 3; see example 10). The plant lines were selected for high levels of expression of each chimeric gene. From each of the selected lines extracellular fluid (EF) (Parent & Asselin, 1984) and total leaf-protein extracts (TE) (Kaufmann et al., 1987) were prepared. Initial dilutions were made of EF and TE of lines 2 and 3 to contain a **chitinase** activity of approximately 2000 cpm (see example 2). The initial dilutions of EF and TE of line 1 were equal to those of line 3. Subsequently, dilution series were made of the initial dilutions and these were tested for antifungal activity. No difference was found in antifungal activity between dilution series of EF and of TE. Moreover the highest antifungal activity was found in the (diluted) extracts of line 2. Apparently, the apoplast-targeted intracellular **glucanase has a synergistic** effect on the antifungal activity of the apoplast-targeted intracellular **chitinase**.

Claims Text - CLTX (13):

13. A plant comprising a recombinant plant genome which genome has been modified so as to include a first expression system for an intracellular plant **chitinase** and a second expression system for an intracellular plant

.beta.-1,3-**glucanase** which when expressed in said plant exhibit a **synergistic** antifungal effect.

Claims Text - CLTX (16):

16. A recombinant plant which exhibits, as a result of expression of one or more recombinant polynucleotides encoding an intracellular plant **chitinase** and a .beta.-1,3-**glucanase,** a relative overexpression of said **chitinase and glucanase** relative to a plant of the same line that does not express said one or more recombinant polynucleotides, wherein said **chitinase and glucanase** in combination exhibit a **synergistic** antifungal effect in the plant.

Claims Text - CLTX (22):

22. A recombinant polynucleotide comprising genetic information for the relative overexpression, relative to a plant of the same line that does not express said recombinant polynucleotide, of a first gene encoding an intracellular plant **chitinase** and a second gene encoding a .beta.-1,3-**glucanase wherein said chitinase and glucanase,** when produced in combination, exhibit a **synergistic** antipathogenic effect in said plant, which polynucleotide comprises:

US-PAT-NO: 5559034

DOCUMENT-IDENTIFIER: US 5559034 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: September 24, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 457552

DATE FILED: June 1, 1995

PARENT-CASE:

This is a divisional application of Ser. No. 08/178,708, filed Jan. 10, 1994, which is a continuation-in-part of Ser. No. 07,505,781, filed Apr. 6, 1990, now abandoned which is a continuation-in-part of Ser. No. 07/104,755, filed Oct. 2, 1987, now abandoned.

US-CL-CURRENT: 435/320.1, 435/252.3 , 435/69.1 , 514/12 , 514/2 , 514/8 , 530/372 , 530/376 , 536/22.1 , 536/23.1 , 536/23.6

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

2 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Brief Summary Text - BSTX (7):

Certain enzymes have also been reported to **synergize** the effect of antifungal agents. Lysozyme has been reported to **synergize** the activity of amphotericin B against Candida albicansand Coccidioides immitis (Collins and Pappagianis (1974) Sabouraudia 12:329-340). Natural mixtures of mycolytic enzymes of fungal origin, designated mycolases, were reported to have a **synergistic** effect on the activity of the antifungal drugs amphotericin B and nystatin (Davies and Pope (1978) Nature 273:235-6; Pope and Davies (1979) Postgraduate Med. J. 55:674-676). The in vitro MICs (minimum inhibitory concentrations) of these antifungal drugs were lowered about 5 to 10-fold in combinations with mycolase. In related in vivo experiments in a mouse model, fungal mycolase was reported to enhance the effectiveness of amphotericin B and nystatin against systemic infection of C. albicans. It was suggested that mycolase, which was suggested to be a mixture of carbohydrases, enhanced penetration of the antibiotic into fungal cells. Fungal mycolases, alone, were described as very effective at releasing protoplasts from Aspergillus fumigatus and C. albicans in vitro and were also reported to have some effect, alone, against systemic fungal infection in the mouse model system. In contrast, a prepared mixture of the carbohydrases **chitinase** (.beta.-1,4 N-acetyl-D-glucosaminidase) and laminarinase (.beta.-1,3(4)**-glucanase**), while reported to effect protoplast release from A. fumigatus and C. albicans, did not enhance the effectiveness of amphotericin B and nystatin in vivo. Recently, in similar in vitro and in vivo experiments with fungal mycolase/amphotericin B mixtures, only slight enhancement of antifungal activity by a fungal mycolase was reported (Chalkley et al. (1985) Sabouraudia 23:147-164). This report suggests that the difference in results compared to those reported earlier by Davies and Pope (supra) may be associated with the lower **chitinase** or lower .beta.-1,6-D**-glucanase** activities in their preparation of mycolase compared to that employed in the previous experiments. The specific enzymatic activities present in fungal mycolases have not been identified, and the specific protein or proteins in mycolase that may effect antibiotic enhancement have not been identified. Some bacterial mycolases have also been reported to effect enhancements (about 2-fold) of the activity of amphotericin B (Oranusi and Trinci (1985) Microbios 43:17-30). Again, no specific enzyme activity was associated with **synergy**.

Brief Summary Text - BSTX (16):

Zeamatin has been isolated in substantially pure form by methods described herein, as demonstrated by the absence of contaminating protein bands in conventional protein gel electrophoresis, as shown in FIG. 4. The N-terminal amino acid sequence (30 amino acids) of zeamatin is provided in Table 5 (SEQ. ID NO: 1). Substantially pure zeamatin displays no detectable **chitinase** activity, 1-3 .beta.**-glucanase,** protease, ribonuclease, phospholipase C,

mannanase, N-.beta.-acetylhexosaminidase or ribosome-inactivating protein activity as assessed by procedures described herein or well known in the art. Substantially pure zeamatin preparations include those in which the 22 kd protein represents about 90% or more of the total protein present in the preparation. In in vitro **synergy** plate assays, zeamatin was found to greatly enhance the anti-Candida activity of nikkomycin X or Z up to about 100 fold, while in liquid culture assays, enhancements of up to 1000 fold were observed. Greater enhancement of anti-Candida activity of nikkomycin X or Z is observed in agar-free medium containing low concentrations of peptone and peptides. Zeamatin also displayed significant enhancement (about 10-fold) of the activity of polyoxin against C. albicans and also enhanced (about 3-fold) the activity of amphotericin B against this yeast

Drawing Description Text - DRTX (3):

FIG. 2 shows elution profiles from CM-Sephadex.TM. column purification of zeamatin from corn protein extracts. A flow rate of 1 ml/min was employed in these separations. Protein in each 6 ml fraction was quantified by measurement of absorbance at 280 nm. Bound protein was eluted with a linear salt gradient (0.01 0.2M NaCl). Four peaks were eluted. FIG. 2A displays the quantitative results of antifungal assays, while FIG. 2B displays the quantitative results of enzyme assays across the four peaks. Absorbance at 280 nm is represented in both A and B by closed circles, solid lines. The results of hyphal extension inhibition of T. reesei (open circles, solid line), hyphal extension inhibition of N. crassa (closed squares, dashed lines) and **synergistic** anti-Candida activity (closed triangles, dotted line) are presented on panel A. The results of **chitinase** (closed triangles, dotted line), **glucanase** (open squares, solid line) and .beta.-N-acetyl-hexosaminidase (closed circles, dashed line) assays are presented in panel B.

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and barley **AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn **AFP** preparations. Growth of the important human pathogen Candida albicanswas found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they **synergized** with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, **synergized with all AFP** preparations, but **synergy** was particularly dramatic with corn-**AFP** preparations. Polyoxin **synergized** significantly with corn and wheat **AFP** preparations, while modest **synergy** was observed with combinations of amphotericin and **AFP** preparations from barley and corn. In contrast, no **synergy** was observed with papulocandin and **AFP** preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-**AFP** preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple

protein peaks (FIG. 1). Using **synergy** with nikkomycin to inhibit the growth of C. albicans as an activity assay, the **synergizing** activity in corn-**AFP** preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (5):

Since a significant loss in specific **synergizing** activity was observed in the conventional phenyl-Sepharose.TM. chromatography step, efforts were made to improve the purification of corn-SAFP activity. Improved purification of zeamatin was obtained by carrying out the CM-Sephadex.TM. chromatography at a slower flow rate than had been employed in previous separations and more importantly, by employing a novel phenyl-Sepharose.TM. chromatographic procedure. Slower elution in the CM-Sephadex.TM. step resulted in four distinct protein peaks (FIG. 2) rather than the three peaks observed previously (FIG. 1). **Synergistic** anti-Candida activity was found only in peak 3. Anti-Neurospora activity was also confined to peak 3, while anti-Trichoderma activity was observed in all peak fractions. All four peaks were also assayed for **chitinase, glucanase** and .beta.-N-acetylhexosaminidase activity. None of these enzyme activities coincided with the anti-Neurospora or **synergistic** anti-Candida activity of peak 3.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases** from Serratia marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in **glucanase** preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified **glucanase** preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both **chitinase and glucanase** called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

Detailed Description Text - DETX (16):

The mechanism by which SAFP **synergizes** the action of polyoxins, nikkomycins and amphotericins is not known. It was thought that SAFP might act to increase penetration of the antibiotics into the target fungi. This could occur as the result of degradation or permeabilization of the fungal cell wall by SAFP. Fungal cell walls are composed of chitin, glucans with .beta.-1,3 or .beta.-1,6- linkages and mannans with .alpha.-1,6, .alpha.-1,2 or

.alpha.-1,3-linkages. It has been demonstrated, however, that zeamatin, unlike other antimycotic agents, does not have **chitinase, glucanase** or mannanase activity. A more probable mechanism, supported by experiments described below, is that SAFP permeabilizes the fungal cell membrane. It is suggested that SAFP lyses fungi by direct insertion of the protein into fungal membranes to form transmembrane pores. Amphiphilic polypeptides may bind to cells through a cationic region of the molecule followed by insertion of a hydrophobic domain through the lipid bilayer of the membrane. For example, zeamatin's amphiphilic nature is suggested by the protein's late elution from CM-Sephadex.TM. (a cationic property) and its retarded passage through phenyl-Sepharose.TM. (a hydrophobic property). That zeamatin acts via cell membrane permeabilization is further supported by the rapid effect of low concentrations of SAFP's on fungi, even at 0.degree. C. For example, 1 g/ml zeamatin induces hyphal rupture in less than 15 seconds at 23.degree. C. This rapid rupture suggests a non-enzymatic mechanism of action. The operability and utility of the SAFPs of the present invention are, however, not dependent upon these suggested mechanisms, and the practice of the present invention does not require characterization of the specific activity of an SAFP. Similarities in structure (similar molecular weights, similar elution behavior on chromatography and homologies in N-terminal sequence) of zeamatin, sormatin and avematin, and more importantly, their common function in **synergism** of anti-fungal activity, indicate that these proteins and other protein strains displaying this function represent a class of proteins (SAFPs) which act by an analogous mechanism.

Detailed Description Text - DETX (34):

A second procedure was found to result in improved purification of zeamatin. Ammonium sulfate fractionation of corn protein extract was performed as described above. The dialyzed 30%-55% fraction was subjected to CM-Sephadex.TM. chromatography, essentially as described above. However, the chromatography was carried out at a slower flow rate (1 ml/min), which resulted in the elution of four distinct peaks (FIG. 2). **Synergistic** anti-Candida activity was confined to peak 3. This peak was also found to contain growth inhibitory activity against Neurospora crassa. Anti-Neurospora activity was found only in corn **AFP** preparations, not in **AFP** preparations of wheat and barley. Anti-Trichoderma activity was found in all four peaks. **Chitinase, glucanase** (.beta.1,3- and .beta.1,6-) and .beta.-N-acetylhexosaminidase activities were also assayed across the four peaks. **Chitinase** was found in all four peaks. A single peak of **glucanase** activity at fraction 47 and a single peak of .beta.-N-acetyl hexosaminidase at fraction 40 were detected. Anti-Neurospora and **synergistic** anti-Candida activity peaked at fraction 44. These antifungal activities did not coincide with any of the enzyme activities tested.

Detailed Description Text - DETX (36):

The three peak fractions from phenyl-Sepharose.TM. chromatography were assayed for enzyme activities and antifungal activities as shown in Table 2. Peak 1 contained most of the **chitinase** activity, a small amount of **glucanase**

activity, and most of the anti-Trichoderma activity. No anti-Neurospora or **synergistic** anti-Candida activity was found in peak 1. Peak 3 contained most of the **glucanase** activity and no other detectable enzymatic or antifungal activity. Peak 2 contained all of the anti-Neurospora and **synergistic** anti-Candida activity and a smaller activity against Trichoderma. Peak 2 contained no detectable **chitinase** activity and a very small amount of **glucanase**.

Detailed Description Text - DETX (56):

Zeamatin partially purified by CM-Sephadex.TM. (fraction CMS) displayed both **chitinase** and .beta.-1, 3 **glucanase** activity in addition to antifungal activity against T. reesi and N. crassa, and **synergistic** activity in combination with antifungalantibiotics, especially nikkomycin, against Candida albicans. Phenyl-Sepharose.TM.-purified zeamatin displayed no **chitinase,** mannanase or .beta.-N-acetylhexosaminidase activity, and little or no **glucanase** activity. **Synergistic** antifungal activity is not associated with the presence of **chitinase or glucanase** activity.

US-PAT-NO: 5521153

DOCUMENT-IDENTIFIER: US 5521153 A

TITLE: Synergistic antifungal protein and compositions containing same

DATE-ISSUED: May 28, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Walden K.	Denver	CO	N/A	N/A
Selitrennikoff; Claude P.	Evergreen	CO	N/A	N/A
Laue; Bridget E.	Davis	CA	N/A	N/A
Potter; Sharon L.	Raleigh	NC	N/A	N/A

APPL-NO: 08/ 178708

DATE FILED: January 10, 1994

PARENT-CASE:

This Application is a continuation-in-part application of U.S. application Ser. No. 07/505,781, filed Apr. 6, 1990, which is a continuation-in-part Application of U.S. application Ser. No. 07/104,755 filed Oct. 2, 1987, both now abandoned. Such applications are herein incorporated by reference .

US-CL-CURRENT: 514/2, 514/12 , 514/8 , 530/372 , 530/376

ABSTRACT:

Novel plant proteins (SAFPs) which synergize the activity of antifungal antibiotics are identified. SAFPs are demonstrated to synergize antifungal antibiotics, such as nikkomycins, polyoxins and amphotericins. SAFPs alone also display antifungal activity against several species of fungi, including strains of Candida, Trichoderma, Neurospora and strains of the plant pathogens Fusarium, Rhizoctonia and Chaetomium. Synergistic antifungal compositions containing SAFP and antifungal antibiotics are provided. In particular, synergistic compositions of corn-SAFP (zeamatin), sorghum-SAFP (sormatin) or oat-SAFP (avematin) and nikkomycin are found to be effective as antifungal compositions, especially against the opportunistic human pathogen Candida albicans. Method for employing SAFPs and synergistic compositions containing them for the inhibition of fungi are provided. In addition, a method for purifying SAFP from grain meal is provided.

15 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

--------- KWIC ---------

Brief Summary Text - BSTX (7):

Certain enzymes have also been reported to **synergize** the effect of antifungal agents. Lysozyme has been reported to **synergize** the activity of amphotericin B against Candida albicans and Coccidioides immitis (Collins and Pappagianis (1974) Sabouraudia 12:329-340). Natural mixtures of mycolytic enzymes of fungal origin, designated mycolases, were reported to have a **synergistic** effect on the activity of the antifungal drugs amphotericin B and nystatin (Davies and Pope (1978) Nature 273:235-6; Pope and Davies (1979) Postgraduate Med. J. 55:674-676). The in vitro MICs (minimum inhibitory concentrations) of these antifungal drugs were lowered about 5 to 10-fold in combinations with mycolase. In related in vivo experiments in a mouse model, fungal mycolase was reported to enhance the effectiveness of amphotericin B and nystatin against systemic infection of C. albicans. It was suggested that mycolase, which was suggested to be a mixture of carbohydrases, enhanced penetration of the antibiotic into fungal cells. Fungal mycolases, alone, were described as very effective at releasing protoplasts from Aspergillus fumigatus and C. albicans in vitro and were also reported to have some effect, alone, against systemic fungal infection in the mouse model system. In contrast, a prepared mixture of the carbohydrases **chitinase** (.beta.-1,4 N-acetyl-D-glucosaminidase) and laminarinase (.beta.-1,3(4)**-glucanase**), while reported to effect protoplast release from A. fumigatus and C. albicans, did not enhance the effectiveness of amphotericin B and nystatin in vivo. Recently, in similar in vitro and in vivo experiments with fungal mycolase/amphotericin B mixtures, only slight enhancement of antifungal activity by a fungal mycolase was reported (Chalkley et al. (1985) Sabouraudia 23:147-164). This report suggests that the difference in results compared to those reported earlier by Davies and Pope (supra) may be associated with the lower **chitinase** or lower .beta.-1,6-D**-glucanase** activities in their preparation of mycolase compared to that employed in the previous experiments. The specific enzymatic activities present in fungal mycolases have not been identified, and the specific protein or proteins in mycolase that may effect antibiotic enhancement have not been identified. Some bacterial mycolases have also been reported to effect enhancements (about 2-fold) of the activity of amphotericin B (Oranusi and Trinci (1985) Microbios 43:17-30). Again, no specific enzyme activity was associated with **synergy**.

Brief Summary Text - BSTX (16):

Zeamatin has been isolated in substantially pure form by methods described herein, as demonstrated by the absence of contaminating protein bands in conventional protein gel electrophoresis, as shown in FIG. 4. The N-terminal amino acid sequence (30 amino acids) of zeamatin is provided in Table 5 [SEQ ID NO:1]. Substantially pure zeamatin displays no detectable **chitinase** activity, 1-3 .beta.**-glucanase,** protease, ribonuclease, phospholipase C, mannanase, N-.beta.-acetylhexosaminidase or ribosome-inactivating protein activity as

assessed by procedures described herein or well known in the art. Substantially pure zeamatin preparations include those in which the 22 kd protein represents about 90% or more of the total protein present in the preparation. In in vitro **synergy** plate assays, zeamatin was found to greatly enhance the anti-Candida activity of nikkomycin X or Z up to about 100 fold, while in liquid culture assays, enhancements of up to 1000 fold were observed. Greater enhancement of anti-Candida activity of nikkomycin X or Z is observed in agar-free medium containing low concentrations of peptone and peptides. Zeamatin also displayed significant enhancement (about 10-fold) of the activity of polyoxin against C. albicans and also enhanced (about 3-fold) the activity of amphotericin B against this yeast.

Drawing Description Text - DRTX (3):

FIG. 2 shows elution profiles from CM-Sephadex.TM. column purification of zeamatin from corn protein extracts. A flow rate of 1 ml/min was employed in these separations. Protein in each 6 ml fraction was quantified by measurement of absorbance at 280 nm. Bound protein was eluted with a linear salt gradient (0.01 0.2M NaCl). Four peaks were eluted. FIG. 2A displays the quantitative results of antifungal assays, while FIG. 2B displays the quantitative results of enzyme assays across the four peaks. Absorbance at 280 nm is represented in both A and B by closed circles, solid lines. The results of hyphal extension inhibition of T. reesei (open circles, solid line), hyphal extension inhibition of N. crassa (closed squares, dashed lines) and **synergistic** anti-Candida activity (closed triangles, dotted line) are presented on panel A. The results of **chitinase** (closed triangles, dotted line), **glucanase** (open squares, solid line) and .beta.-N-acetyl-hexosaminidase (closed circles, dashed line) assays are presented in panel B.

Detailed Description Text - DETX (4):

The present work is an extension of experiments with antifungal proteins (AFPs) which were isolated from barley, corn and wheat (Roberts and Selitrennikoff (1988) J. Gen. Microbiol. 134:169-176). These proteins inhibited growth of Trichoderma, Phycomyces and Alternaria and have been shown to have endochitinase activity. Wheat and barley **AFP chitinases** did not inhibit growth of Neurospora, in contrast to corn **AFP** preparations. Growth of the important human pathogen Candida albicans was found to be resistant to inhibition by the AFPs in agar plate assays. AFPs were then assessed to determine if they **synergized** with antifungal antibiotics to lower the MICs of the antibiotics. Selected results of such experiments are summarized in Table 1. Nikkomycin, a mixture of nikkomycin Z and X, **synergized with all AFP** preparations, but **synergy** was particularly dramatic with corn-**AFP** preparations. Polyoxin **synergized** significantly with corn and wheat **AFP** preparations, while modest **synergy** was observed with combinations of amphotericin and **AFP** preparations from barley and corn. In contrast, no **synergy** was observed with papulocandin and **AFP** preparations. Wheat and barley AFPs (Table 1) were purified to homogeneity. The corn-**AFP** preparation (Table 1) when chromatographed through a CM-Sephadex.TM. column was shown to contain multiple protein peaks (FIG. 1). Using **synergy** with nikkomycin to inhibit the growth of

C. albicans as an activity assay, the **synergizing** activity in corn-**AFP** preparations was found to reside in a single protein fraction from CM-Sephadex.TM. column chromatography, see FIG. 1. Further purification of this fraction using conventional hydrophobic column chromatography with phenyl-Sepharose.TM. resulted in the isolation of an approximately 22 kd protein. The 22 kd protein which effected strong enhancement of nikkomycin activity was designated a corn-SAFP, and specifically named zeamatin.

Detailed Description Text - DETX (5):

Since a significant loss in specific **synergizing** activity was observed in the conventional phenyl-Sepharose.TM. chromatography step, efforts were made to improve the purification of corn-SAFP activity. Improved purification of zeamatin was obtained by carrying out the CM-Sephadex.TM. chromatography at a slower flow rate than had been employed in previous separations and more importantly, by employing a novel phenyl-Sepharose.TM. chromatographic procedure. Slower elution in the CM-Sephadex.TM. step resulted in four distinct protein peaks (FIG. 2) rather than the three peaks observed previously (FIG. 1). **Synergistic** anti-Candida activity was found only in peak 3. Anti-Neurospora activity was also confined to peak 3, while anti-Trichoderma activity was observed in all peak fractions. All four peaks were also assayed for **chitinase, glucanase** and .beta.-N-acetylhexosaminidase activity. None of these enzyme activities coincided with the anti-Neurospora or **synergistic** anti-Candida activity of peak 3.

Detailed Description Text - DETX (12):

Chitinase and glucanase preparations from several other sources were also tested in the **synergy** assay. No **synergy** with nikkomycin was found with **chitinases** from Serratia marcescens, Pseudomonas stuzeri, or Streptomyces griseus or in **glucanase** preparations from Penicillium or mollusk. Significant **synergy** was observed, however, with a partially purified **glucanase** preparation from the fungus Rhizopus and in commercial bacterial (Arthrobacter luteus) enzyme mixture containing both **chitinase and glucanase** called Zymolase (available from Sigma Chemical Co., St. Louis, Mo.). The nature of the **synergizing** enzymes in these preparations has not been identified, and it is not known whether they act by a mechanism that is similar to plant SAFPs. The **synergizing** activity in these preparations may be due to minor components in the mixtures.

Detailed Description Text - DETX (16):

The mechanism by which SAFP **synergizes** the action of polyoxins, nikkomycins and amphotericins is not known. It was thought that SAFP might act to increase penetration of the antibiotics into the target fungi. This could occur as the result of degradation or permeabilization of the fungal cell wail by SAFP. Fungal cell walls are composed of chitin, glucans with .beta.-1,3 or .beta.-1,6- linkages and mannans with .alpha.-1,6, .alpha.-1,2 or .alpha.-1,3-linkages. It has been demonstrated, however, that zeamatin, unlike

other antimycotic agents, does not have **chitinase, glucanase** or mannanase activity. A more probable mechanism, supported by experiments described below, is that SAFP permeabilizes the fungal cell membrane. It is suggested that SAFP lyses fungi by direct insertion of the protein into fungal membranes to form transmembrane pores. Amphiphilic polypeptides may bind to cells through a cationic region of the molecule followed by insertion of a hydrophobic domain through the lipid bilayer of the membrane. For example, zeamatin's amphiphilic nature is suggested by the protein's late elution from CM-Sephadex.TM. (a cationic property) and its retarded passage through phenyl-Sepharose.TM. (a hydrophobic property). That zeamatin acts via cell membrane permeabilization is further supported by the rapid effect of low concentrations of SAFP's on fungi, even at 0.degree. C. For example, 1 g/ml zeamatin induces hyphal rupture in less than 15 seconds at 23.degree. C. This rapid rupture suggests a non-enzymatic mechanism of action. The operability and utility of the SAFPs of the present invention are, however, not dependent upon these suggested mechanisms, and the practice of the present invention does not require characterization of the specific activity of an SAFP. Similarities in structure (similar molecular weights, similar elution behavior on chromatography and homologies in N-terminal sequence) of zeamatin, sormatin and avematin, and more importantly, their common function in **synergism** of anti-fungal activity, indicate that these proteins and other protein strains displaying this function represent a class of proteins (SAFPs) which act by an analogous mechanism.

Detailed Description Text - DETX (34):

A second procedure was found to result in improved purification of zeamatin. Ammonium sulfate fractionation of corn protein extract was performed as described above. The dialyzed 30%-55% fraction was subjected to CM-Sephadex.TM. chromatography, essentially as described above. However, the chromatography was carried out at a slower flow rate (1 ml/min), which resulted in the elution of four distinct peaks (FIG. 2). **Synergistic** anti-Candida activity was confined to peak 3. This peak was also found to contain growth inhibitory activity against Neurospora crassa. Anti-Neurospora activity was found only in corn **AFP** preparations, not in **AFP** preparations of wheat and barley. Anti-Trichoderma activity was found in all four peaks. **Chitinase, glucanase** (.beta.1,3- and .beta.1,6-) and .beta.-N-acetylhexosaminidase activities were also assayed across the four peaks. **Chitinase** was found in all four peaks. A single peak of **glucanase** activity at fraction 47 and a single peak of .beta.-N-acetyl hexosaminidase at fraction 40 were detected. Anti-Neurospora and **synergistic** anti-Candida activity peaked at fraction 44. These antifungal activities did not coincide with any of the enzyme activities tested.

Detailed Description Text - DETX (36):

The three peak fractions from phenyl-Sepharose.TM. chromatography were assayed for enzyme activities and antifungal activities as shown in Table 2. Peak 1 contained most of the **chitinase** activity, a small amount of **glucanase** activity, and most of the anti-Trichoderma activity. No anti-Neurospora or

synergistic anti-Candida activity was found in peak 1. Peak 3 contained most of the **glucanase** activity and no other detectable enzymatic or antifungal activity. Peak 2 contained all of the anti-Neurospora and **synergistic** anti-Candida activity and a smaller activity against Trichoderma. Peak 2 contained no detectable **chitinase** activity and a very small amount of **glucanase**.

Detailed Description Text - DETX (56):

Zeamatin partially purified by CM-Sephadex.TM. (fraction CMS) displayed both **chitinase** and .beta.-1,3 **glucanase** activity in addition to antifungal activity against T. reesi and N. crassa, and **synergistic** activity in combination with antifungalantibiotics, especially nikkomycin, against Candida albicans. Phenyl-Sepharose.TM.-purified zeamatin displayed no **chitinase,** mannanase or .beta.-N-acetylhexosaminidase activity, and little or no **glucanase** activity. **Synergistic** antifungal activity is not associated with the presence of **chitinase or glucanase** activity.

US-PAT-NO: 5399680

DOCUMENT-IDENTIFIER: US 5399680 A

TITLE: Rice chitinase promoter

DATE-ISSUED: March 21, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zhu; Qun	San Diego	CA	N/A	N/A
Lamb; Christopher J.	San Diego	CA	N/A	N/A

APPL-NO: 07/ 704288

DATE FILED: May 22, 1991

US-CL-CURRENT: 536/24.1, 435/418 , 435/419 , 435/69.1 , 435/91.3

ABSTRACT:

Novel chitinase gene, and its associated regulatory region, from a monocotyledon plant is described.

13 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (5):

Chitinase (EC 3.2.1.14) catalyzes the hydrolysis of the .beta.-1,4 linkages of the N-acetyl-D-glucosamine polymer chitin. Chitin does not occur in higher plants, but is present in the cell walls of many fungi. **Chitinase,** which exhibits complex developmental and hormonal regulation, has been found in many species of higher plants. In addition, **chitinase** activity is markedly increased by wounding, ethylene, or microbial elicitors. Furthermore, **chitinase** is involved in the hypersensitive resistance response to microbial attack. Purified plant **chitinase** attacks and partially digests isolated cell walls of potentially pathogenic fungi. It is this latter enzyme activity, rather than chitin-binding lectin activity, that is responsible for the inhibition of fungal growth. **Chitinase** and .beta.**-glucanase** exhibit **synergistic** antifungal activity in vitro. A number of pathogenesis-related proteins (also referred to as "PR proteins") have been found to be **chitinases**

or glucanases.

US-PAT-NO: 4032663

DOCUMENT-IDENTIFIER: US 4032663 A

TITLE: Process for using cell wall-lysing enzymes

DATE-ISSUED: June 28, 1977

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kobayashi; Reisuke	Shizuoka	N/A	N/A	JA
Sato; Hironari	Shimizu	N/A	N/A	JA
Takita; Kiyoshi	Shimizu	N/A	N/A	JA
Toyama; Nobuo	Miyazaki	N/A	N/A	JA

APPL-NO: 05/ 675448

DATE FILED: April 9, 1976

PARENT-CASE:

This is a division of application Ser. No. 522,304, filed Nov. 8, 1974, now U.S. Pat. No. 3,969,189, issued July 13, 1976, which in turn is a continuation of application Ser. No. 314,933 filed Dec. 14, 1972, now U.S. Pat. No. 3,890,198.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JA	46-100700	December 14, 1971
JA	47-9486	January 27, 1972

US-CL-CURRENT: 426/51, 426/61 , 435/200 , 435/201 , 435/203 , 435/209 , 435/223 , 435/839 , 435/853 , 435/911 , 435/917 , 435/921 , 435/922 , 435/923 , 435/933 , 435/942

ABSTRACT:

Complex enzymes which can lyse the cell wall of a variety of microorganisms such as bacteria, fungi, yeast, Basidiomycetes and chlorella are produced and recovered from cultivation of Pellicularia sasakii or Pellicularia filamentosa.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 5

---------- KWIC ---------

Brief Summary Text - BSTX (33):

It is considered that the cell wall-lysing complex enzyme produced by Pellicularia sasakii and the complex enzyme produced by Pellicularia filamentosa are each a mixture of cellulase, **glucanase, chitinase,** protease, hemi-cellulase and carboxymethyl cellulase in the form of a complex and exhibit their high activity of lysing the cell of various microorganisms through the **synergistic** effect of the actions of the particular component enzymes, though it is presumed that these complex enzymes produced by the different species of Pellicularia have compositions which are more or less different from each other, as it is observed that these complex enzymes show more or less different properties and potencies for the particular enzymatic activities.

US-PAT-NO: 3969189

DOCUMENT-IDENTIFIER: US 3969189 A

TITLE: Cell wall-lysing complex enzymes and a process for the production thereof

DATE-ISSUED: July 13, 1976

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kobayashi; Reisuke	Shizuoka	N/A	N/A	JA
Sato; Hironari	Shimizu	N/A	N/A	JA
Takita; Kiyoshi	Shimizu	N/A	N/A	JA
Toyama; Nobuo	Miyazaki	N/A	N/A	JA

DISCLAIMER DATE: 19920617

APPL-NO: 05/ 522304

DATE FILED: November 8, 1974

PARENT-CASE:

This is a continuation of application Ser. No. 314,933, filed Dec. 14, 1972, now U.S. Pat. No. 3,890,198.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JA	46-100700	December 14, 1971
JA	47-9486	January 27, 1972

US-CL-CURRENT: 435/206, 426/51 , 435/200 , 435/209 , 435/267 , 435/911

ABSTRACT:

Complex enzymes which can lyse the cell wall of a variety of microorganisms such as bacteria, fungi, yeast, Basidiomycetes and chlorella are produced and recovered from cultivation of Pellicularia sasakii or Pellicularia filamentosa.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (34):

It is considered that the cell wall-lysing complex enzyme produced by Pellicularia sasakii and the complex enzyme produced by Pellicularia filamentosa are each a mixture of cellulase, **glucanase, chitinase,** protease, hemi-cellulase and carboxymethyl cellulase in the form of a complex and exhibit their high activity of lysing the cell of various microorganisms through the **synergistic** effect of the actions of the particular component enzymes, though it is presumed that these complex enzymes produced by the different species of Pellicularia have compositions which are more or less different from each other, as it is observed that these complex enzymes show more or less different properties and potencies for the particular enzymatic activities.

US-PAT-NO: 3890198

DOCUMENT-IDENTIFIER: US 3890198 A

TITLE: Cell wall-lysing complex enzymes and a process for the production thereof

DATE-ISSUED: June 17, 1975

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kobayashi; Reisuke	Shimizu	N/A	N/A	JA
Sato; Hironari	Shimizu	N/A	N/A	JA
Takita; Kiyoshi	Shimizu	N/A	N/A	JA
Toyama; Nobuo	Miyazaki	N/A	N/A	JA

APPL-NO: 05/ 314933

DATE FILED: December 14, 1972

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JA	46-100700	December 14, 1971
JA	47-9486	January 27, 1972

US-CL-CURRENT: 435/206, 435/259 , 435/814 , 435/816 , 435/911

ABSTRACT:

Complex enzymes which can lyse the cell wall of a variety of microorganisms such as bacteria, fungi, yeast, Basidiomycetes and chlorella are produced and recovered from cultivation of Pellicularia sasakii or Pellicularia filamentosa.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (33):

It is considered that the cell wall-lysing complex enzyme produced by Pellicularia sasakii and the complex enzyme produced by Pellicularia filamentosa are each a mixture of cellulase, **glucanase, chitinase,** protease, hemi-cellulase and carboxymethyl cellulase in the form of a complex and exhibit their high activity of lysing the cell of various microorganisms through the **synergistic** effect of the actions of the particular component enzymes, though it is presumed that these complex enzymes produced by the different species of

Pellicularia have compositions which are more or less different from each other, as it is observed that these complex enzymes show more or less different properties and potencies for the particular enzymatic activities.

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  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:15:20 ON 25 APR 2003

=> fil .bec,caba,agricola
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS, CABA, AGRICOLA'
ENTERED AT 16:15:45 ON 25 APR 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

13 FILES IN THE FILE LIST

=> s chitinase#
FILE 'MEDLINE'
L1        1508 CHITINASE#

FILE 'SCISEARCH'
L2        3055 CHITINASE#

FILE 'LIFESCI'
L3        1459 CHITINASE#

FILE 'BIOTECHDS'
L4         906 CHITINASE#

FILE 'BIOSIS'
L5        3587 CHITINASE#

FILE 'EMBASE'
L6        1127 CHITINASE#

FILE 'HCAPLUS'
L7        4250 CHITINASE#

FILE 'NTIS'
L8          30 CHITINASE#

FILE 'ESBIOBASE'
L9        1275 CHITINASE#

FILE 'BIOTECHNO'
L10       1285 CHITINASE#

FILE 'WPIDS'
L11        420 CHITINASE#

FILE 'CABA'
L12       2130 CHITINASE#

FILE 'AGRICOLA'
L13       1273 CHITINASE#

TOTAL FOR ALL FILES
L14      22305 CHITINASE#

=> s barley
FILE 'MEDLINE'
L15       4771 BARLEY

FILE 'SCISEARCH'
```

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L16      28135 BARLEY

FILE 'LIFESCI'
L17       6036 BARLEY

FILE 'BIOTECHDS'
L18       1684 BARLEY

FILE 'BIOSIS'
L19      43479 BARLEY

FILE 'EMBASE'
L20       3342 BARLEY

FILE 'HCAPLUS'
L21      45121 BARLEY

FILE 'NTIS'
L22       1163 BARLEY

FILE 'ESBIOBASE'
L23       5852 BARLEY

FILE 'BIOTECHNO'
L24       4061 BARLEY

FILE 'WPIDS'
L25       5705 BARLEY

FILE 'CABA'
L26      65919 BARLEY

FILE 'AGRICOLA'
L27      28518 BARLEY

TOTAL FOR ALL FILES
L28     243786 BARLEY

=> s glucanase#
FILE 'MEDLINE'
L29       1385 GLUCANASE#

FILE 'SCISEARCH'
L30       3104 GLUCANASE#

FILE 'LIFESCI'
L31       1468 GLUCANASE#

FILE 'BIOTECHDS'
L32       1181 GLUCANASE#

FILE 'BIOSIS'
L33       4058 GLUCANASE#

FILE 'EMBASE'
L34       1116 GLUCANASE#

FILE 'HCAPLUS'
L35       5429 GLUCANASE#

FILE 'NTIS'
L36         33 GLUCANASE#

FILE 'ESBIOBASE'
```

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L37       1050 GLUCANASE#

FILE 'BIOTECHNO'
L38       1204 GLUCANASE#

FILE 'WPIDS'
L39        665 GLUCANASE#

FILE 'CABA'
L40       2075 GLUCANASE#

FILE 'AGRICOLA'
L41       1459 GLUCANASE#

TOTAL FOR ALL FILES
L42      24227 GLUCANASE#

=> s psi or protein synthesis inhibit?
FILE 'MEDLINE'
          5882 PSI
       1150658 PROTEIN
        356504 SYNTHESIS
       1024204 INHIBIT?
          7439 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L43      13301 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'SCISEARCH'
         17039 PSI
        985068 PROTEIN
        612830 SYNTHESIS
        812008 INHIBIT?
          2914 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L44      19946 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'LIFESCI'
          3016 PSI
        397806 "PROTEIN"
         96158 "SYNTHESIS"
        283170 INHIBIT?
          1399 PROTEIN SYNTHESIS INHIBIT?
                 ("PROTEIN"(W)"SYNTHESIS"(W)INHIBIT?)
L45       4407 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'BIOTECHDS'
           626 PSI
         99519 PROTEIN
         18625 SYNTHESIS
         40363 INHIBIT?
            83 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L46         707 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'BIOSIS'
          8597 PSI
       1289272 PROTEIN
        499476 SYNTHESIS
       1110166 INHIBIT?
          5894 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L47      14472 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'EMBASE'
```

```
          5722 PSI
       1101189 "PROTEIN"
        466036 "SYNTHESIS"
        915463 INHIBIT?
          6676 PROTEIN SYNTHESIS INHIBIT?
                 ("PROTEIN"(W)"SYNTHESIS"(W)INHIBIT?)
L48      12383 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'HCAPLUS'
         55103 PSI
       1503055 PROTEIN
       1055796 SYNTHESIS
       1566155 INHIBIT?
          5004 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L49      60095 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'NTIS'
          6890 PSI
         11863 PROTEIN
         34157 SYNTHESIS
         19761 INHIBIT?
            49 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L50       6939 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'ESBIOBASE'
          3730 PSI
        453542 PROTEIN
        121210 SYNTHESIS
        326227 INHIBIT?
          1476 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L51       5201 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'BIOTECHNO'
          2856 PSI
        567049 PROTEIN
        135400 SYNTHESIS
        278347 INHIBIT?
          3338 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L52       6187 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'WPIDS'
         11093 PSI
         95960 PROTEIN
         68631 SYNTHESIS
        192363 INHIBIT?
           110 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L53      11201 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'CABA'
          3429 PSI
        298823 PROTEIN
         73159 SYNTHESIS
        193896 INHIBIT?
           644 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L54       4072 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'AGRICOLA'
           889 PSI
```

```
        124145 PROTEIN
         37286 SYNTHESIS
         61280 INHIBIT?
           359 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L55       1247 PSI OR PROTEIN SYNTHESIS INHIBIT?

TOTAL FOR ALL FILES
L56     160158 PSI OR PROTEIN SYNTHESIS INHIBIT?

=> s afp or antifungal protein
FILE 'MEDLINE'
          5282 AFP
         24037 ANTIFUNGAL
       1150658 PROTEIN
            92 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L57       5354 AFP OR ANTIFUNGAL PROTEIN

FILE 'SCISEARCH'
          2999 AFP
         13630 ANTIFUNGAL
        985068 PROTEIN
           165 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L58       3141 AFP OR ANTIFUNGAL PROTEIN

FILE 'LIFESCI'
           719 AFP
          8758 "ANTIFUNGAL"
        397806 "PROTEIN"
            52 ANTIFUNGAL PROTEIN
                 ("ANTIFUNGAL"(W)"PROTEIN")
L59        760 AFP OR ANTIFUNGAL PROTEIN

FILE 'BIOTECHDS'
           146 AFP
           988 ANTIFUNGAL
         99519 PROTEIN
            21 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L60        165 AFP OR ANTIFUNGAL PROTEIN

FILE 'BIOSIS'
          4654 AFP
         30442 ANTIFUNGAL
       1289272 PROTEIN
           168 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L61       4796 AFP OR ANTIFUNGAL PROTEIN

FILE 'EMBASE'
          4599 AFP
         21177 "ANTIFUNGAL"
       1101189 "PROTEIN"
            72 ANTIFUNGAL PROTEIN
                 ("ANTIFUNGAL"(W)"PROTEIN")
L62       4658 AFP OR ANTIFUNGAL PROTEIN

FILE 'HCAPLUS'
          3210 AFP
         21489 ANTIFUNGAL
       1503055 PROTEIN
           242 ANTIFUNGAL PROTEIN
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```
                (ANTIFUNGAL(W)PROTEIN)
L63        3415 AFP OR ANTIFUNGAL PROTEIN

FILE 'NTIS'
             76 AFP
            127 ANTIFUNGAL
          11863 PROTEIN
              0 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L64           76 AFP OR ANTIFUNGAL PROTEIN

FILE 'ESBIOBASE'
           1017 AFP
           4090 ANTIFUNGAL
         453542 PROTEIN
             97 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L65        1098 AFP OR ANTIFUNGAL PROTEIN

FILE 'BIOTECHNO'
           1276 AFP
           3524 ANTIFUNGAL
         567049 PROTEIN
             64 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L66        1324 AFP OR ANTIFUNGAL PROTEIN

FILE 'WPIDS'
            258 AFP
           8823 ANTIFUNGAL
          95960 PROTEIN
             21 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L67         278 AFP OR ANTIFUNGAL PROTEIN

FILE 'CABA'
            251 AFP
          22840 ANTIFUNGAL
         298823 PROTEIN
             90 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L68         326 AFP OR ANTIFUNGAL PROTEIN

FILE 'AGRICOLA'
             47 AFP
           3253 ANTIFUNGAL
         124145 PROTEIN
             45 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L69          80 AFP OR ANTIFUNGAL PROTEIN

TOTAL FOR ALL FILES
L70       25471 AFP OR ANTIFUNGAL PROTEIN

=> s l14(8a)(serratia or marcescens)
FILE 'MEDLINE'
           6420 SERRATIA
           4985 MARCESCENS
L71          74 L1 (8A)(SERRATIA OR MARCESCENS)

FILE 'SCISEARCH'
           3625 SERRATIA
           2805 MARCESCENS
L72          91 L2 (8A)(SERRATIA OR MARCESCENS)
```

```
FILE 'LIFESCI'
          2297 SERRATIA
          1706 MARCESCENS
L73         60 L3 (8A)(SERRATIA OR MARCESCENS)

FILE 'BIOTECHDS'
           968 SERRATIA
           562 MARCESCENS
L74         72 L4 (8A)(SERRATIA OR MARCESCENS)

FILE 'BIOSIS'
          8885 SERRATIA
          6297 MARCESCENS
L75        121 L5 (8A)(SERRATIA OR MARCESCENS)

FILE 'EMBASE'
          6525 SERRATIA
          4423 MARCESCENS
L76         60 L6 (8A)(SERRATIA OR MARCESCENS)

FILE 'HCAPLUS'
          7733 SERRATIA
          5856 MARCESCENS
L77        180 L7 (8A)(SERRATIA OR MARCESCENS)

FILE 'NTIS'
           144 SERRATIA
           114 MARCESCENS
L78          2 L8 (8A)(SERRATIA OR MARCESCENS)

FILE 'ESBIOBASE'
           852 SERRATIA
           601 MARCESCENS
L79         44 L9 (8A)(SERRATIA OR MARCESCENS)

FILE 'BIOTECHNO'
          1533 SERRATIA
          1130 MARCESCENS
L80         55 L10(8A)(SERRATIA OR MARCESCENS)

FILE 'WPIDS'
          1111 SERRATIA
           466 MARCESCENS
L81         10 L11(8A)(SERRATIA OR MARCESCENS)

FILE 'CABA'
          1286 SERRATIA
           718 MARCESCENS
L82         47 L12(8A)(SERRATIA OR MARCESCENS)

FILE 'AGRICOLA'
           493 SERRATIA
           296 MARCESCENS
L83         26 L13(8A)(SERRATIA OR MARCESCENS)

TOTAL FOR ALL FILES
L84        842 L14(8A)(SERRATIA OR MARCESCENS)

=> s l14(8a)l28
FILE 'MEDLINE'
L85         20 L1 (8A)L15

FILE 'SCISEARCH'
```

```
L86         44 L2 (8A)L16

FILE 'LIFESCI'
L87         15 L3 (8A)L17

FILE 'BIOTECHDS'
L88          7 L4 (8A)L18

FILE 'BIOSIS'
L89         52 L5 (8A)L19

FILE 'EMBASE'
L90         11 L6 (8A)L20

FILE 'HCAPLUS'
L91         71 L7 (8A)L21

FILE 'NTIS'
L92          1 L8 (8A)L22

FILE 'ESBIOBASE'
L93         19 L9 (8A)L23

FILE 'BIOTECHNO'
L94         16 L10(8A)L24

FILE 'WPIDS'
L95          2 L11(8A)L25

FILE 'CABA'
L96         37 L12(8A)L26

FILE 'AGRICOLA'
L97         14 L13(8A)L27

TOTAL FOR ALL FILES
L98        309 L14(8A) L28

=> s l42(8a)l28
FILE 'MEDLINE'
L99         66 L29(8A)L15

FILE 'SCISEARCH'
L100       164 L30(8A)L16

FILE 'LIFESCI'
L101        32 L31(8A)L17

FILE 'BIOTECHDS'
L102        34 L32(8A)L18

FILE 'BIOSIS'
L103       235 L33(8A)L19

FILE 'EMBASE'
L104        35 L34(8A)L20

FILE 'HCAPLUS'
L105       365 L35(8A)L21

FILE 'NTIS'
L106         0 L36(8A)L22

FILE 'ESBIOBASE'
```

```
L107        42 L37(8A)L23

FILE 'BIOTECHNO'
L108        51 L38(8A)L24

FILE 'WPIDS'
L109        12 L39(8A)L25

FILE 'CABA'
L110       191 L40(8A)L26

FILE 'AGRICOLA'
L111        86 L41(8A)L27

TOTAL FOR ALL FILES
L112      1313 L42(8A) L28

=> s l56(8a)l28
FILE 'MEDLINE'
L113        22 L43(8A)L15

FILE 'SCISEARCH'
L114        32 L44(8A)L16

FILE 'LIFESCI'
L115        14 L45(8A)L17

FILE 'BIOTECHDS'
L116         3 L46(8A)L18

FILE 'BIOSIS'
L117        52 L47(8A)L19

FILE 'EMBASE'
L118        10 L48(8A)L20

FILE 'HCAPLUS'
L119        62 L49(8A)L21

FILE 'NTIS'
L120         0 L50(8A)L22

FILE 'ESBIOBASE'
L121        10 L51(8A)L23

FILE 'BIOTECHNO'
L122        15 L52(8A)L24

FILE 'WPIDS'
L123         0 L53(8A)L25

FILE 'CABA'
L124        58 L54(8A)L26

FILE 'AGRICOLA'
L125        16 L55(8A)L27

TOTAL FOR ALL FILES
L126       294 L56(8A) L28

=> s l70(8a)(aspergillus or giganteus)
FILE 'MEDLINE'
         21045 ASPERGILLUS
           215 GIGANTEUS
```

```
L127        19 L57(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'SCISEARCH'
         21362 ASPERGILLUS
           753 GIGANTEUS
L128        21 L58(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'LIFESCI'
         11420 ASPERGILLUS
           318 GIGANTEUS
L129        10 L59(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'BIOTECHDS'
          8521 ASPERGILLUS
            43 GIGANTEUS
L130         4 L60(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'BIOSIS'
         37425 ASPERGILLUS
          1684 GIGANTEUS
L131        21 L61(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'EMBASE'
         16456 ASPERGILLUS
           179 GIGANTEUS
L132        15 L62(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'HCAPLUS'
         41802 ASPERGILLUS
           623 GIGANTEUS
L133        22 L63(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'NTIS'
           218 ASPERGILLUS
            30 GIGANTEUS
L134         0 L64(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'ESBIOBASE'
          5850 ASPERGILLUS
           192 GIGANTEUS
L135        16 L65(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'BIOTECHNO'
          7215 ASPERGILLUS
           113 GIGANTEUS
L136        16 L66(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'WPIDS'
          5725 ASPERGILLUS
            20 GIGANTEUS
L137         3 L67(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'CABA'
         24356 ASPERGILLUS
           598 GIGANTEUS
L138        13 L68(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'AGRICOLA'
         11968 ASPERGILLUS
           256 GIGANTEUS
L139        11 L69(8A)(ASPERGILLUS OR GIGANTEUS)

TOTAL FOR ALL FILES
L140       171 L70(8A)(ASPERGILLUS OR GIGANTEUS)
```

```
=> s (184 and (198 or l112 or l126 or l140)) or (198 and (l112 or l126 or l140)) or
(l112 and (l126 or l140)) or (l126 and l140)
FILE 'MEDLINE'
L141          2 (L71 AND (L85 OR L99 OR L113 OR L127)) OR (L85 AND (L99 OR L113
                OR L127)) OR (L99 AND (L113 OR L127)) OR (L113 AND L127)

FILE 'SCISEARCH'
L142         10 (L72 AND (L86 OR L100 OR L114 OR L128)) OR (L86 AND (L100 OR
                L114 OR L128)) OR (L100 AND (L114 OR L128)) OR (L114 AND L128)

FILE 'LIFESCI'
L143          4 (L73 AND (L87 OR L101 OR L115 OR L129)) OR (L87 AND (L101 OR
                L115 OR L129)) OR (L101 AND (L115 OR L129)) OR (L115 AND L129)

FILE 'BIOTECHDS'
L144          1 (L74 AND (L88 OR L102 OR L116 OR L130)) OR (L88 AND (L102 OR
                L116 OR L130)) OR (L102 AND (L116 OR L130)) OR (L116 AND L130)

FILE 'BIOSIS'
L145         11 (L75 AND (L89 OR L103 OR L117 OR L131)) OR (L89 AND (L103 OR
                L117 OR L131)) OR (L103 AND (L117 OR L131)) OR (L117 AND L131)

FILE 'EMBASE'
L146          1 (L76 AND (L90 OR L104 OR L118 OR L132)) OR (L90 AND (L104 OR
                L118 OR L132)) OR (L104 AND (L118 OR L132)) OR (L118 AND L132)

FILE 'HCAPLUS'
L147         16 (L77 AND (L91 OR L105 OR L119 OR L133)) OR (L91 AND (L105 OR
                L119 OR L133)) OR (L105 AND (L119 OR L133)) OR (L119 AND L133)

FILE 'NTIS'
L148          0 (L78 AND (L92 OR L106 OR L120 OR L134)) OR (L92 AND (L106 OR
                L120 OR L134)) OR (L106 AND (L120 OR L134)) OR (L120 AND L134)

FILE 'ESBIOBASE'
L149          5 (L79 AND (L93 OR L107 OR L121 OR L135)) OR (L93 AND (L107 OR
                L121 OR L135)) OR (L107 AND (L121 OR L135)) OR (L121 AND L135)

FILE 'BIOTECHNO'
L150          4 (L80 AND (L94 OR L108 OR L122 OR L136)) OR (L94 AND (L108 OR
                L122 OR L136)) OR (L108 AND (L122 OR L136)) OR (L122 AND L136)

FILE 'WPIDS'
L151          2 (L81 AND (L95 OR L109 OR L123 OR L137)) OR (L95 AND (L109 OR
                L123 OR L137)) OR (L109 AND (L123 OR L137)) OR (L123 AND L137)

FILE 'CABA'
L152          9 (L82 AND (L96 OR L110 OR L124 OR L138)) OR (L96 AND (L110 OR
                L124 OR L138)) OR (L110 AND (L124 OR L138)) OR (L124 AND L138)

FILE 'AGRICOLA'
L153          1 (L83 AND (L97 OR L111 OR L125 OR L139)) OR (L97 AND (L111 OR
                L125 OR L139)) OR (L111 AND (L125 OR L139)) OR (L125 AND L139)

TOTAL FOR ALL FILES
L154         66 (L84 AND (L98 OR L112 OR L126 OR L140)) OR (L98 AND (L112 OR
                L126 OR L140)) OR (L112 AND (L126 OR L140)) OR (L126 AND L140)

=> s (l14 and (142 or l56 or l70)) or (142 and (l56 or l70)) or (l56 and l70)
FILE 'MEDLINE'
L155        174 (L1 AND (L29 OR L43 OR L57)) OR (L29 AND (L43 OR L57)) OR (L43
                AND L57)
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FILE 'SCISEARCH'
L156       710 (L2 AND (L30 OR L44 OR L58)) OR (L30 AND (L44 OR L58)) OR (L44
               AND L58)

FILE 'LIFESCI'
L157       248 (L3 AND (L31 OR L45 OR L59)) OR (L31 AND (L45 OR L59)) OR (L45
               AND L59)

FILE 'BIOTECHDS'
L158       145 (L4 AND (L32 OR L46 OR L60)) OR (L32 AND (L46 OR L60)) OR (L46
               AND L60)

FILE 'BIOSIS'
L159       782 (L5 AND (L33 OR L47 OR L61)) OR (L33 AND (L47 OR L61)) OR (L47
               AND L61)

FILE 'EMBASE'
L160       112 (L6 AND (L34 OR L48 OR L62)) OR (L34 AND (L48 OR L62)) OR (L48
               AND L62)

FILE 'HCAPLUS'
L161       843 (L7 AND (L35 OR L49 OR L63)) OR (L35 AND (L49 OR L63)) OR (L49
               AND L63)

FILE 'NTIS'
L162         2 (L8 AND (L36 OR L50 OR L64)) OR (L36 AND (L50 OR L64)) OR (L50
               AND L64)

FILE 'ESBIOBASE'
L163       256 (L9 AND (L37 OR L51 OR L65)) OR (L37 AND (L51 OR L65)) OR (L51
               AND L65)

FILE 'BIOTECHNO'
L164       218 (L10 AND (L38 OR L52 OR L66)) OR (L38 AND (L52 OR L66)) OR (L52
               AND L66)

FILE 'WPIDS'
L165       114 (L11 AND (L39 OR L53 OR L67)) OR (L39 AND (L53 OR L67)) OR (L53
               AND L67)

FILE 'CABA'
L166       570 (L12 AND (L40 OR L54 OR L68)) OR (L40 AND (L54 OR L68)) OR (L54
               AND L68)

FILE 'AGRICOLA'
L167       304 (L13 AND (L41 OR L55 OR L69)) OR (L41 AND (L55 OR L69)) OR (L55
               AND L69)

TOTAL FOR ALL FILES
L168      4478 (L14 AND (L42 OR L56 OR L70)) OR (L42 AND (L56 OR L70)) OR (L56
               AND L70)

=> s l168 and synerg?
FILE 'MEDLINE'
         72658 SYNERG?
L169         9 L155 AND SYNERG?

FILE 'SCISEARCH'
         45632 SYNERG?
L170        21 L156 AND SYNERG?

FILE 'LIFESCI'
         16863 SYNERG?
L171        13 L157 AND SYNERG?
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FILE 'BIOTECHDS'
         1333 SYNERG?
L172       13 L158 AND SYNERG?

FILE 'BIOSIS'
        53583 SYNERG?
L173       23 L159 AND SYNERG?

FILE 'EMBASE'
        41463 SYNERG?
L174        6 L160 AND SYNERG?

FILE 'HCAPLUS'
        85341 SYNERG?
L175       37 L161 AND SYNERG?

FILE 'NTIS'
         3255 SYNERG?
L176        0 L162 AND SYNERG?

FILE 'ESBIOBASE'
        16311 SYNERG?
L177        9 L163 AND SYNERG?

FILE 'BIOTECHNO'
        15110 SYNERG?
L178        9 L164 AND SYNERG?

FILE 'WPIDS'
        20164 SYNERG?
L179        7 L165 AND SYNERG?

FILE 'CABA'
        13243 SYNERG?
L180       16 L166 AND SYNERG?

FILE 'AGRICOLA'
         4355 SYNERG?
L181       11 L167 AND SYNERG?

TOTAL FOR ALL FILES
L182      174 L168 AND SYNERG?

=> s l168 and transgen?
FILE 'MEDLINE'
        42952 TRANSGEN?
L183       16 L155 AND TRANSGEN?

FILE 'SCISEARCH'
        63539 TRANSGEN?
L184      133 L156 AND TRANSGEN?

FILE 'LIFESCI'
        23632 TRANSGEN?
L185       23 L157 AND TRANSGEN?

FILE 'BIOTECHDS'
        20788 TRANSGEN?
L186       63 L158 AND TRANSGEN?

FILE 'BIOSIS'
        59005 TRANSGEN?
L187       59 L159 AND TRANSGEN?
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FILE 'EMBASE'
        34183 TRANSGEN?
L188        8 L160 AND TRANSGEN?

FILE 'HCAPLUS'
        57340 TRANSGEN?
L189       89 L161 AND TRANSGEN?

FILE 'NTIS'
          577 TRANSGEN?
L190        0 L162 AND TRANSGEN?

FILE 'ESBIOBASE'
        28849 TRANSGEN?
L191       23 L163 AND TRANSGEN?

FILE 'BIOTECHNO'
        29823 TRANSGEN?
L192       26 L164 AND TRANSGEN?

FILE 'WPIDS'
         9079 TRANSGEN?
L193       30 L165 AND TRANSGEN?

FILE 'CABA'
        22121 TRANSGEN?
L194       51 L166 AND TRANSGEN?

FILE 'AGRICOLA'
        12363 TRANSGEN?
L195       27 L167 AND TRANSGEN?

TOTAL FOR ALL FILES
L196      548 L168 AND TRANSGEN?

=> s (l154 or l182 or l196) not 1994-1997/py
FILE 'MEDLINE'
      1675196 1994-1997/PY
L197       19 (L141 OR L169 OR L183) NOT 1994-1997/PY

FILE 'SCISEARCH'
      3498768 1994-1997/PY
L198       94 (L142 OR L170 OR L184) NOT 1994-1997/PY

FILE 'LIFESCI'
       440456 1994-1997/PY
L199       18 (L143 OR L171 OR L185) NOT 1994-1997/PY

FILE 'BIOTECHDS'
        60613 1994-1997/PY
L200       37 (L144 OR L172 OR L186) NOT 1994-1997/PY

FILE 'BIOSIS'
      2227913 1994-1997/PY
L201       59 (L145 OR L173 OR L187) NOT 1994-1997/PY

FILE 'EMBASE'
      1515306 1994-1997/PY
L202        9 (L146 OR L174 OR L188) NOT 1994-1997/PY

FILE 'HCAPLUS'
      3040839 1994-1997/PY
L203       87 (L147 OR L175 OR L189) NOT 1994-1997/PY
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FILE 'NTIS'
        163629 1994-1997/PY
L204         0 (L148 OR L176 OR L190) NOT 1994-1997/PY

FILE 'ESBIOBASE'
        765969 1994-1997/PY
L205        23 (L149 OR L177 OR L191) NOT 1994-1997/PY

FILE 'BIOTECHNO'
        410147 1994-1997/PY
L206        22 (L150 OR L178 OR L192) NOT 1994-1997/PY

FILE 'WPIDS'
       2383049 1994-1997/PY
L207        24 (L151 OR L179 OR L193) NOT 1994-1997/PY

FILE 'CABA'
        616931 1994-1997/PY
L208        49 (L152 OR L180 OR L194) NOT 1994-1997/PY

FILE 'AGRICOLA'
        295235 1994-1997/PY
L209        16 (L153 OR L181 OR L195) NOT 1994-1997/PY

TOTAL FOR ALL FILES
L210       457 (L154 OR L182 OR L196) NOT 1994-1997/PY

=> s l210 not 1998-2000/py
FILE 'MEDLINE'
       1384553 1998-2000/PY
L211        13 L197 NOT 1998-2000/PY

FILE 'SCISEARCH'
       2912462 1998-2000/PY
L212        60 L198 NOT 1998-2000/PY

FILE 'LIFESCI'
        334799 1998-2000/PY
L213        12 L199 NOT 1998-2000/PY

FILE 'BIOTECHDS'
         42738 1998-2000/PY
L214        30 L200 NOT 1998-2000/PY

FILE 'BIOSIS'
       1689771 1998-2000/PY
L215        40 L201 NOT 1998-2000/PY

FILE 'EMBASE'
       1302504 1998-2000/PY
L216         5 L202 NOT 1998-2000/PY

FILE 'HCAPLUS'
       2674252 1998-2000/PY
L217        50 L203 NOT 1998-2000/PY

FILE 'NTIS'
         73418 1998-2000/PY
L218         0 L204 NOT 1998-2000/PY

FILE 'ESBIOBASE'
        853099 1998-2000/PY
L219        12 L205 NOT 1998-2000/PY
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FILE 'BIOTECHNO'
        355338 1998-2000/PY
L220         13 L206 NOT 1998-2000/PY

FILE 'WPIDS'
       2478093 1998-2000/PY
L221          7 L207 NOT 1998-2000/PY

FILE 'CABA'
        494844 1998-2000/PY
L222         35 L208 NOT 1998-2000/PY

FILE 'AGRICOLA'
        213793 1998-2000/PY
L223          9 L209 NOT 1998-2000/PY

TOTAL FOR ALL FILES
L224        286 L210 NOT 1998-2000/PY

=> s l224 not 2001-2003/py
FILE 'MEDLINE'
       1176976 2001-2003/PY
L225          5 L211 NOT 2001-2003/PY

FILE 'SCISEARCH'
       2141013 2001-2003/PY
L226         32 L212 NOT 2001-2003/PY

FILE 'LIFESCI'
        197014 2001-2003/PY
L227          5 L213 NOT 2001-2003/PY

FILE 'BIOTECHDS'
         41589 2001-2003/PY
L228         16 L214 NOT 2001-2003/PY

FILE 'BIOSIS'
       1142122 2001-2003/PY
L229         17 L215 NOT 2001-2003/PY

FILE 'EMBASE'
        979363 2001-2003/PY
L230          2 L216 NOT 2001-2003/PY

FILE 'HCAPLUS'
       2265194 2001-2003/PY
L231         21 L217 NOT 2001-2003/PY

FILE 'NTIS'
         30635 2001-2003/PY
L232          0 L218 NOT 2001-2003/PY

FILE 'ESBIOBASE'
        628246 2001-2003/PY
L233          0 L219 NOT 2001-2003/PY

FILE 'BIOTECHNO'
        254287 2001-2003/PY
L234          1 L220 NOT 2001-2003/PY

FILE 'WPIDS'
       2071705 2001-2003/PY
L235          0 L221 NOT 2001-2003/PY
```

FILE 'CABA'
303908 2001-2003/PY
L236 21 L222 NOT 2001-2003/PY

FILE 'AGRICOLA'
102562 2001-2003/PY
L237 5 L223 NOT 2001-2003/PY

TOTAL FOR ALL FILES
L238 125 L224 NOT 2001-2003/PY

=> dup rem l238
PROCESSING COMPLETED FOR L238
L239 73 DUP REM L238 (52 DUPLICATES REMOVED)

=> d tot

L239 ANSWER 1 OF 73 CABA COPYRIGHT 2003 CABI
TI New strategies for obtaining fungal-resistant plants.
SO Developing agricultural biotechnology in the Netherlands, (1993) pp. 44.
Publisher: Centre for Agricultural Publishing and Documentation (PUDOC).
ISBN: 90-220-1081-3
AU Cornelissen, B. J. C.; Kan, J. A. L. van; Van Kan, J. A. L.; Vuijk, D. H. [EDITOR]; Dekkers, J. J. [EDITOR]; Plas, H. C. van der (Editors) [EDITOR]
AN 95:161696 CABA

L239 ANSWER 2 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI PHYSIOLOGICAL COMPENSATION IN ANTISENSE TRANSFORMANTS - SPECIFIC INDUCTION OF AN ERSATZ GLUCAN ENDO-1,3-BETA-GLUCOSIDASE IN PLANTS INFECTED WITH NECROTIZING VIRUSES
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (01 OCT 1993) Vol. 90, No. 19, pp. 8792-8796.
ISSN: 0027-8424.
AU BEFFA R S; NEUHAUS J M; MEINS F (Reprint)
AN 93:615638 SCISEARCH

L239 ANSWER 3 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI INCREASED TOLERANCE TO 2 OOMYCETE PATHOGENS IN **TRANSGENIC** TOBACCO EXPRESSING PATHOGENESIS-RELATED PROTEIN-1A
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (01 AUG 1993) Vol. 90, No. 15, pp. 7327-7331.
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AN 93:488457 SCISEARCH

L239 ANSWER 4 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 1
TI ONLY SPECIFIC TOBACCO (NICOTIANA-TABACUM) **CHITINASES** AND BETA-1,3-**GLUCANASES** EXHIBIT ANTIFUNGAL ACTIVITY
SO PLANT PHYSIOLOGY, (MAR 1993) Vol. 101, No. 3, pp. 857-863.
ISSN: 0032-0889.
AU SELABUURLAGE M B (Reprint); PONSTEIN A S; BRESVLOEMANS S A; MELCHERS L S; VANDENELZEN P J M; CORNELISSEN B J C
AN 93:175245 SCISEARCH

L239 ANSWER 5 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Strategies for control of fungal diseases with **transgenic** plants;
transgenic plant with fungus disease-resistance
SO Plant Physiol.; (1993) 101, 3, 709-12
CODEN: PLPHAY
AU Cornelissen B J C; Melchers L S
AN 1993-05093 BIOTECHDS

L239 ANSWER 6 OF 73 MEDLINE DUPLICATE 3
TI Plant 'pathogenesis-related' proteins and their role in defense against pathogens.
SO BIOCHIMIE, (1993) 75 (8) 687-706. Ref: 227
Journal code: 1264604. ISSN: 0300-9084.
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L239 ANSWER 7 OF 73 MEDLINE DUPLICATE 4
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Journal code: 9106343. ISSN: 0167-4412.
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AN 93192519 MEDLINE

L239 ANSWER 8 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI PLANT **CHITINASES** AND THEIR ROLES IN RESISTANCE TO FUNGAL DISEASES
SO JOURNAL OF NEMATOLOGY, (DEC 1993) Vol. 25, No. 4, pp. 526-540.
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AU PUNJA Z K (Reprint); ZHANG Y Y
AN 94:40807 SCISEARCH

L239 ANSWER 9 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI AN ACIDIC CLASS-III **CHITINASE** IN SUGAR-BEET - INDUCTION BY CERCOSPORA-BETICOLA, CHARACTERIZATION, AND EXPRESSION IN **TRANSGENIC** TOBACCO PLANTS
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ISSN: 0894-0282.
AU NIELSEN K K (Reprint); MIKKELSEN J D; KRAGH K M; BOJSEN K
AN 93:581844 SCISEARCH

L239 ANSWER 10 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI PATHOGEN, SALICYLIC-ACID AND DEVELOPMENTAL DEPENDENT EXPRESSION OF A BETA-1,3-**GLUCANASE** GUS GENE FUSION IN **TRANSGENIC** TOBACCO PLANTS
SO PLANT JOURNAL, (SEP 1993) Vol. 4, No. 3, pp. 481-493.
ISSN: 0960-7412.
AU HENNIG J; DEWEY R E; CUTT J R; KLESSIG D F (Reprint)
AN 93:560628 SCISEARCH

L239 ANSWER 11 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI ANALYSIS OF REGULATORY ELEMENTS INVOLVED IN STRESS-INDUCED AND ORGAN-SPECIFIC EXPRESSION OF TOBACCO ACIDIC AND BASIC BETA-1,3-**GLUCANASE** GENES
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ISSN: 0167-4412.
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L239 ANSWER 12 OF 73 CABA COPYRIGHT 2003 CABI
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L239 ANSWER 13 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
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AN 1993:511939 BIOSIS

L239 ANSWER 14 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI CYTOLOGY OF INFECTION OF 35S-BEAN **CHITINASE TRANSGENIC** CANOLA PLANTS BY RHIZOCTONIA-SOLANI - CYTOCHEMICAL ASPECTS OF CHITIN BREAKDOWN IN-VIVO
SO PLANT JOURNAL, (AUG 1993) Vol. 4, No. 2, pp. 295-305.
ISSN: 0960-7412.
AU BENHAMOU N (Reprint); BROGLIE K; CHET I; BROGLIE R
AN 93:524966 SCISEARCH

L239 ANSWER 15 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 6
TI VIRUS AND FUNGAL RESISTANCE - FROM LABORATORY TO FIELD
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L239 ANSWER 16 OF 73 CABA COPYRIGHT 2003 CABI
TI Virus and fungal resistance: from laboratory to field.
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AN 95:37486 CABA

L239 ANSWER 17 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI ACCUMULATION OF PATHOGENESIS-RELATED PROTEINS IN THE EPIDERMIS OF TOMATO LEAVES INFECTED BY CLADOSPORIUM-FULVUM
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AU WUBBEN J P (Reprint); EIJKELBOOM C A; DEWIT P J G M
AN 94:344625 SCISEARCH

L239 ANSWER 18 OF 73 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 7
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CODEN: PMBIDB; ISSN: 0167-4412
AU Duering, Klaus
AN 1994:4555 HCAPLUS
DN 120:4555

L239 ANSWER 19 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Disease-resistance: development of a gene transfer system for sunflower

(Helianthus annuus L.) somatic embryos;
somatic embryogenesis and propagation for potential **transgenic** plant production with Sclerotinia sclerotiorum disease-resistance (conference abstract)
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CODEN: PPPMA9
AU Hearn S J; Webster J R
AN 1994-07506 BIOTECHDS

L239 ANSWER 20 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Expression of hydrolases in **transgenic** alfalfa plants; **chitinase**, cellulase and cellobiohydrolase gene expression in **transgenic** plant for fungus disease-resistance and crop improvement (conference abstract)
SO Plant Physiol.; (1993) 102, 1, Suppl., 167
CODEN: PLPHAY
AU Masoud S; Lamb C J; Dixon R A
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L239 ANSWER 21 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI A 61 BP ENHANCER ELEMENT OF THE TOBACCO BETA-1,3-**GLUCANASE** B-GENE INTERACTS WITH ONE OR MORE REGULATED NUCLEAR PROTEINS
SO PLANT MOLECULAR BIOLOGY, (JAN 1993) Vol. 21, No. 1, pp. 121-131.
ISSN: 0167-4412.
AU HART C M; NAGY F; MEINS F (Reprint)
AN 93:80503 SCISEARCH

L239 ANSWER 22 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 8
TI **GLUCANASE**, GLUCAN SYNTHASE AND **CHITINASE** ACTIVITY IN **BARLEY** GENOTYPES SUSCEPTIBLE OR RESISTANT TO ERYSIPHE-GRAMINIS F SP HORDEI
SO BIOLOGIA PLANTARUM, (1993) Vol. 35, No. 1, pp. 95-101.
ISSN: 0006-3134.
AU FRIC F (Reprint); HUTTOVA J
AN 93:319421 SCISEARCH

L239 ANSWER 23 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Emerging strategies for enhancing crop resistance to microbial pathogens; engineering disease-resistance in **transgenic** plant for crop improvement (conference paper)
SO Curr.Plant Sci.Biotechnol.Agric.; (1993) 45-60
CODEN: 9999T
AU Lamb C J
AN 1993-14530 BIOTECHDS

L239 ANSWER 24 OF 73 CABA COPYRIGHT 2003 CABI
TI The biology of plants transformed with chimeric class I **chitinase** and beta -1,3-**glucanase** genes.
SO (1993) pp. 27. Centro de Reuniones Internacionales Sobre Biologia No. 10. 7 ref. Publisher: Instituto Juan March de Estudios e Investigaciones. Meeting Info.: Workshop on engineering plants against pests and pathogens,11-13 January 1993 Madrid, Spain. ISBN: 84-7919-509-8
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AN 94:31881 CABA

L239 ANSWER 25 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 10
TI PLANT ENZYMES WITH ANTIMICROBIAL PROPERTIES
SO PHYTOPROTECTION, (APR 1993) Vol. 74, No. 1, pp. 3-18.
ISSN: 0031-9511.
AU ASSELIN A (Reprint)
AN 93:508700 SCISEARCH

L239 ANSWER 26 OF 73 CABA COPYRIGHT 2003 CABI

TI [Plant enzymes with antimicrobial properties].
Quelques enzymes vegetales a potentiel antimicrobien.
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AU Asselin, A.
AN 94:52204 CABA

L239 ANSWER 27 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI SEQUENCE VARIATION, DIFFERENTIAL EXPRESSION AND CHROMOSOMAL LOCATION OF RICE **CHITINASE** GENES
SO MOLECULAR & GENERAL GENETICS, (OCT 1993) Vol. 241, No. 1-2, pp. 1-10. ISSN: 0026-8925.
AU NISHIZAWA Y (Reprint); KISHIMOTO N; SAITO A; HIBI T
AN 93:620432 SCISEARCH

L239 ANSWER 28 OF 73 CABA COPYRIGHT 2003 CABI
TI [Behavioural studies of chemical interactions between bees and plants: application to the evaluation of the effect of **transgenic** rapeseed (Brassica napus var. oleifera) on the honey bee (Apis mellifera L.)].
Etudes comportementales des interactions chimiques abeille-plante: application a l'evaluation de l'impact de colzas **transgeniques** (Brassica napus var. oleifera) sur l'abeille domestique (Apis mellifera L.).
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AU Picard-Nizou, A.-L.
AN 94:60340 CABA

L239 ANSWER 29 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI SUPPRESSION OF BETA-1,3-**GLUCANASE TRANSGENE** EXPRESSION IN HOMOZYGOUS PLANTS
SO EMBO JOURNAL, (JUL 1992) Vol. 11, No. 7, pp. 2595-2602. ISSN: 0261-4189.
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AN 92:399584 SCISEARCH

L239 ANSWER 30 OF 73 MEDLINE DUPLICATE 11
TI The function of vacuolar beta-1,3-**glucanase** investigated by antisense transformation. Susceptibility of **transgenic** Nicotiana sylvestris plants to Cercospora nicotianae infection.
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AU Neuhaus J M; Flores S; Keefe D; Ahl-Goy P; Meins F Jr
AN 92353389 MEDLINE

L239 ANSWER 31 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI DIFFERENTIAL ACCUMULATION OF MESSENGER-RNAS ENCODING EXTRACELLULAR AND INTRACELLULAR PR PROTEINS IN TOMATO INDUCED BY VIRULENT AND AVIRULENT RACES OF CLADOSPORIUM-FULVUM
SO PLANT MOLECULAR BIOLOGY, (NOV 1992) Vol. 20, No. 3, pp. 513-527. ISSN: 0167-4412.
AU VANKAN J A L (Reprint); JOOSTEN M H A J; WAGEMAKERS C A M; VANDENBERGVELTHUIS G C M; DEWIT P J G M
AN 92:639586 SCISEARCH

L239 ANSWER 32 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI THE STRUCTURE AND REGULATION OF HOMEOLOGOUS TOBACCO ENDOCHITINASE GENES OF NICOTIANA-SYLVESTRIS AND N-TOMENTOSIFORMIS ORIGIN
SO MOLECULAR & GENERAL GENETICS, (APR 1992) Vol. 232, No. 3, pp. 460-469. ISSN: 0026-8925.
AU VANBUUREN M; NEUHAUS J M; SHINSHI H; RYALS J; MEINS F (Reprint)

AN 92:300862 SCISEARCH

L239 ANSWER 33 OF 73 HCAPLUS COPYRIGHT 2003 ACS
TI Antifungal activity of chitin-binding PR-4 type proteins from barley grain and stressed leaf
SO FEBS Letters (1992), 307(3), 389-92
CODEN: FEBLAL; ISSN: 0014-5793
AU Hejgaard, Joern; Jacobsen, Susanne; Bjoern, Soeren E.; Kragh, Karsten M.
AN 1992:567867 HCAPLUS
DN 117:167867

L239 ANSWER 34 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI ETHYLENE-INDUCED **CHITINASE** AND BETA-1,3-**GLUCANASE** ACCUMULATE SPECIFICALLY IN THE LOWER EPIDERMIS AND ALONG VASCULAR STRANDS OF BEAN-LEAVES
SO PLANTA, (FEB 1992) Vol. 186, No. 3, pp. 367-375.
ISSN: 0032-0935.
AU MAUCH F (Reprint); MEEHL J B; STAEHELIN L A
AN 92:121419 SCISEARCH

L239 ANSWER 35 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI EXTRACELLULAR TARGETED VACUOLAR PR-PROTEINS EXHIBIT ANTIFUNGAL ACTIVITY IN THE EXTRA CELLULAR FLUID OF **TRANSGENIC** PLANTS.
SO KEYSTONE SYMPOSIUM ON CROP IMPROVEMENT VIA BIOTECHNOLOGY: AN INTERNATIONAL PERSPECTIVE, KEYSTONE, COLORADO, USA, APRIL 10-16, 1992. J CELL BIOCHEM SUPPL. (1992) 0 (16 PART F), 223.
CODEN: JCBSD7.
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AN 1992:336444 BIOSIS

L239 ANSWER 36 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI REGULATED INACTIVATION OF HOMOLOGOUS GENE-EXPRESSION IN **TRANSGENIC** NICOTIANA-SYLVESTRIS PLANTS CONTAINING A DEFENSE-RELATED TOBACCO **CHITINASE** GENE
SO MOLECULAR & GENERAL GENETICS, (NOV 1992) Vol. 235, No. 2-3, pp. 179-188.
ISSN: 0026-8925.
AU HART C M; FISCHER B; NEUHAUS J M; MEINS F (Reprint)
AN 92:698734 SCISEARCH

L239 ANSWER 37 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI SUBCELLULAR-LOCALIZATION OF PLANT **CHITINASES** AND 1,3-BETA-**GLUCANASES** IN CLADOSPORIUM-FULVUM (SYN FULVIA-FULVA)-INFECTED TOMATO LEAVES
SO PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, (JUL 1992) Vol. 41, No. 1, pp. 23-32.
ISSN: 0885-5765.
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AN 92:676780 SCISEARCH

L239 ANSWER 38 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Purification and characterization of Cellvibrio mixtus beta-1,3-**glucanase** from recombinant E. coli;
recombinant endo-1,3-beta-D-**glucanase** expression in Escherichia coli; may be used as fungicide and for insect resistance in **transgenic** plant (conference abstract)
SO Aust.Microbiologist; (1992) 13, 3, A22
AU Sakellaris H; Pemberton J M; Manners J M
AN 1993-01539 BIOTECHDS

L239 ANSWER 39 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Induction of defence protein in plant;
protease-inhibitor gene expression by treatment with jasmonic acid; improved insect resistance and virus disease-resistance in e.g.

transgenic plant
AN 1992-03343 BIOTECHDS
PI WO 9118512 12 Dec 1991

L239 ANSWER 40 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI DNA sequence encoding vacuole targeting peptide; especially signal peptide of tobacco **chitinase** or **glucanase** gene
AN 1992-02734 BIOTECHDS
PI EP 462065 18 Dec 1991

L239 ANSWER 41 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Anti-pathogenic compositions; comprise beta-1,3-**glucanase** and **chitinase**; gene expression in **transgenic** plant for improved insect resistance, or fungus or nematode disease-resistance
AN 1991-14849 BIOTECHDS
PI EP 448511 25 Sep 1991

L239 ANSWER 42 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Plants with improved resistance to pathogenic fungus; **transgenic** plant expressing **chitinase** and/or beta-1,3-**glucanase** genes modified for tissue-specific gene expression in apoplast and improved disease-resistance; DNA sequence
AN 1991-12262 BIOTECHDS
PI EP 440304 7 Aug 1991

L239 ANSWER 43 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New DNA regulatory sequence from new tobacco **chitinase** gene; used to increase expression of foreign gene in **transgenic** plant; DNA sequence; potential application in improved herbicide resistance
AN 1991-06911 BIOTECHDS
PI EP 418695 27 Mar 1991

L239 ANSWER 44 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI A SHORT C-TERMINAL SEQUENCE IS NECESSARY AND SUFFICIENT FOR THE TARGETING OF **CHITINASES** TO THE PLANT VACUOLE
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1991) Vol. 88, No. 22, pp. 10362-10366.
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AN 91:635803 SCISEARCH

L239 ANSWER 45 OF 73 MEDLINE DUPLICATE 12
TI Biochemical and molecular characterization of three barley seed proteins with antifungal properties.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1991 Jan 25) 266 (3) 1564-73. Journal code: 2985121R. ISSN: 0021-9258.
AU Leah R; Tommerup H; Svendsen I; Mundy J
AN 91107649 MEDLINE

L239 ANSWER 46 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF 3 BARLEY SEED PROTEINS WITH ANTIFUNGAL PROPERTIES
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1991) Vol. 266, No. 3, pp. 1564-1573.
AU LEAH R; TOMMERUP H; SVENDSEN I; MUNDY J (Reprint)
AN 91:58988 SCISEARCH

L239 ANSWER 47 OF 73 LIFESCI COPYRIGHT 2003 CSA
TI Biochemical and molecular characterization of three barley seed proteins with antifungal properties.
SO J. BIOL. CHEM., (1991) vol. 266, no. 3, pp. 1564-1573.
AU Leach, R.; Tommerup, H.; Svendsen, I.; Mundy, J.
AN 91:49415 LIFESCI

L239 ANSWER 48 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI DIFFERENTIAL INDUCTION OF ACQUIRED-RESISTANCE AND PR GENE-EXPRESSION IN TOBACCO BY VIRUS-INFECTION, ETHEPHON TREATMENT, UV-LIGHT AND WOUNDING
SO PLANT MOLECULAR BIOLOGY, (1991) Vol. 17, No. 6, pp. 1117-1125.
AU BREDERODE F T; LINTHORST H J M (Reprint); BOL J F
AN 91:638475 SCISEARCH

L239 ANSWER 49 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI DEVELOPMENTAL AND PATHOGEN-INDUCED ACTIVATION OF THE ARABIDOPSIS ACIDIC **CHITINASE** PROMOTER
SO PLANT CELL, (1991) Vol. 3, No. 10, pp. 1063-1072.
AU SAMAC D A (Reprint); SHAH D M
AN 91:598095 SCISEARCH

L239 ANSWER 50 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 13
TI HIGH-LEVEL EXPRESSION OF A TOBACCO **CHITINASE** GENE IN NICOTIANA-SYLVESTRIS - SUSCEPTIBILITY OF **TRANSGENIC** PLANTS TO CERCOSPORA-NICOTIANAE INFECTION
SO PLANT MOLECULAR BIOLOGY, (1991) Vol. 16, No. 1, pp. 141-151.
AU NEUHAUS J M; AHLGOY P; HINZ U; FLORES S; MEINS F (Reprint)
AN 91:68535 SCISEARCH

L239 ANSWER 51 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 14
TI PURIFICATION AND CHARACTERIZATION OF 3 CHITINASES AND ONE BETA-1,3-GLUCANASE ACCUMULATING IN THE MEDIUM OF CELL-SUSPENSION CULTURES OF BARLEY (HORDEUM-VULGARE L)
SO PLANT SCIENCE, (1991) Vol. 76, No. 1, pp. 65-77.
AU KRAGH K M (Reprint); JACOBSEN S; MIKKELSEN J D; NIELSEN K A
AN 91:363435 SCISEARCH

L239 ANSWER 52 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Disease-resistant **transgenic** plant;
disease-resistance; cucumber or tobacco pathogenesis-related protein, e.g. peroxidase, **chitinase**, lysozyme, etc. gene cloning and expression in tobacco; DNA sequence
AN 1991-00910 BIOTECHDS
PI EP 392225 17 Oct 1990

L239 ANSWER 53 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI CLONING OF 2 GENES FROM BACILLUS-CIRCULANS WL-12 WHICH ENCODE 1,3-BETA-**GLUCANASE** ACTIVITY
SO JOURNAL OF GENERAL MICROBIOLOGY, (1990) Vol. 136, No. DEC, pp. 2377-2383.
AU FISKE M J (Reprint); TOBEYFINCHER K L; FUCHS R L
AN 91:26366 SCISEARCH

L239 ANSWER 54 OF 73 LIFESCI COPYRIGHT 2003 CSA DUPLICATE 16
TI Zeamatin, an **antifungal protein** from maize with membrane-permeabilizing activity.
SO J. GEN. MICROBIOL., (1990) vol. 136, no. 9, pp. 1771-1778.
AU Roberts, W.K.; Selitrennikoff, C.P.
AN 90:49586 LIFESCI

L239 ANSWER 55 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI TISSUE-SPECIFIC AND PATHOGEN-INDUCED REGULATION OF A NICOTIANA-PLUMBAGINIFOLIA BETA-1,3-**GLUCANASE** GENE
SO PLANT CELL, (1990) Vol. 2, No. 12, pp. 1131-1143.
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AN 91:2472 SCISEARCH

L239 ANSWER 56 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Barley hydrolases and ribosome-inactivating proteins inhibit fungal growth;

chitinase, ribosome-inactivating protein and endo-1,3-beta-D-**glucanase** gene cloning; potential use in improved disease-resistance of **transgenic** plant (conference paper)
SO Eur.Congr.Biotechnol.; (1990) 5 Meet., 916-18
AU Tommerup H; Leah R; Jensen A B; Logeman J; Mundy J
AN 1992-00924 BIOTECHDS

L239 ANSWER 57 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI **Chitinase** and **glucanase** from fungally-infected alfalfa;
disease-resistance gene cloning and expression in **transgenic** plant (conference abstract)
SO J.Cell.Biochem.; (1990) Suppl.14E, 322
CODEN: JCEBD5
AU Maher E A; Dixon R A
AN 1990-14265 BIOTECHDS

L239 ANSWER 58 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI PLANT PATHOGENESIS-RELATED PROTEINS INDUCED BY VIRUS INFECTION.
SO COOK, R. J. (ED.). ANNUAL REVIEW OF PHYTOPATHOLOGY, VOL. 28. X+493P. ANNUAL REVIEWS INC.: PALO ALTO, CALIFORNIA, USA. ILLUS. MAPS. (1990) 0 (0), 113-138.
CODEN: APPYAG. ISSN: 0066-4286. ISBN: 0-8243-1328-3.
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AN 1990:504650 BIOSIS

L239 ANSWER 59 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 17
TI GEOGRAPHIC VARIATION OF ALPHA AMYLASE BETA AMYLASE BETA **GLUCANASE** PULLULANASE AND **CHITINASE** ACTIVITY IN GERMINATING HORDEUM-SPONTANEUM **BARLEY** FROM ISRAEL AND JORDAN.
SO GENETICA (DORDR), (1990) 82 (2), 73-78.
CODEN: GENEA3. ISSN: 0016-6707.
AU AHOKAS H; NASKALI L
AN 1991:163449 BIOSIS

L239 ANSWER 60 OF 73 SCISEARCH COPYRIGHT 2003 ISI (R)
TI GEOGRAPHIC-VARIATION OF ALPHA-AMYLASE, BETA-AMYLASE, BETA-**GLUCANASE**, PULLULANASE AND **CHITINASE** ACTIVITY IN GERMINATING HORDEUM-SPONTANEUM **BARLEY** FROM ISRAEL AND JORDAN
SO GENETICA, (1990) Vol. 82, No. 2, pp. 73-78.
AU AHOKAS H (Reprint); NASKALI L
AN 91:49634 SCISEARCH

L239 ANSWER 61 OF 73 HCAPLUS COPYRIGHT 2003 ACS
TI Variation of .alpha.-amylase, .beta.-amylase, .beta.-glucanase, pullulanase, proteinase and chitinase activity in germinated samples of the wild progenitor of barley
SO Journal of the Institute of Brewing (1990), 96(1), 27-31
CODEN: JINBAL; ISSN: 0368-2587
AU Ahokas, Hannu; Naskali, Leena
AN 1990:115802 HCAPLUS
DN 112:115802

L239 ANSWER 62 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Chemical induction of cloned genes in plants;
DNA sequence of pathogenesis-related protein gene, vector and processes for inducible expression leading to herbicide resistance, insect resistance in **transgenic** plant
AN 1989-14435 BIOTECHDS
PI EP 332104 13 Sep 1989

L239 ANSWER 63 OF 73 HCAPLUS COPYRIGHT 2003 ACS

TI **Synergistic antifungal protein**, its preparation from corn, compositions containing it, and its use
SO PCT Int. Appl., 56 pp.
CODEN: PIXXD2
IN Roberts, Walden K.; Selitrennikoff, Claude P.; Laue, Bridget E.
AN 1990:30639 HCAPLUS
DN 112:30639

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 8902744	A1	19890406	WO 1988-US3420	19881003
W: AU, DK, FI, JP, KR, NO				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
AU 8825572	A1	19890418	AU 1988-25572	19881003

L239 ANSWER 64 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Influence of a plant transit peptide on bacterial **chitinase** activity in tobacco;
Streptomyces plicatus gene cloning and expression; **transgenic** plant construction; callus culture (conference abstract)
SO J.Cell.Biochem.; (1989) Suppl.13D, 267
CODEN: JCEBD5
AU Passiatore J E; Cannon F; Buchanan-Wollaston V
AN 1989-12626 BIOTECHDS

L239 ANSWER 65 OF 73 LIFESCI COPYRIGHT 2003 CSA DUPLICATE 18
TI Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of **chitinase** and beta -1,3-**glucanase**.
SO PLANT PHYSIOL., (1988) vol. 88, no. 3, pp. 936-942.
AU Mauch, F.; Mauch-Mani, B.; Boller, T.
AN 88:83206 LIFESCI

L239 ANSWER 66 OF 73 HCAPLUS COPYRIGHT 2003 ACS
TI Plant and bacterial chitinases differ in antifungal activity
SO Journal of General Microbiology (1988), 134(1), 169-76
CODEN: JGMIAN; ISSN: 0022-1287
AU Roberts, Walden K.; Selitrennikoff, Claude P.
AN 1988:164591 HCAPLUS
DN 108:164591

L239 ANSWER 67 OF 73 HCAPLUS COPYRIGHT 2003 ACS
TI A modified procedure for the assay of enzyme activity hydrolyzing the insoluble polysaccharides of the yeast cell wall
SO Biotekhnologiya (1987), 3(4), 514-19
CODEN: BTKNEZ; ISSN: 0234-2758
AU Mosin, V. A.; Vorotilo, S. P.; Savchenkov, S. N.; Konovalov, S. A.
AN 1987:613877 HCAPLUS
DN 107:213877

L239 ANSWER 68 OF 73 MEDLINE
TI Effect of mycolase and amphotericin B on Candida albicans and Candida pseudotropicalis in vitro and in vivo.
SO SABOURAUDIA, (1985 Jun) 23 (3) 147-64.
Journal code: 0417341. ISSN: 0036-2174.
AU Chalkley L J; Trinci A P; Pope A M
AN 85272894 MEDLINE

L239 ANSWER 69 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 19
TI LYTICASE ENDO **GLUCANASE** AND PROTEASE ACTIVITIES THAT ACT TOGETHER IN YEAST OERSKOVIA-XANTHINEOLYTICA CELL LYSIS.
SO J BACTERIOL, (1980) 142 (2), 414-423.
CODEN: JOBAAY. ISSN: 0021-9193.
AU SCOTT J H; SCHEKMAN R
AN 1980:260822 BIOSIS

L239 ANSWER 70 OF 73 HCAPLUS COPYRIGHT 2003 ACS
TI Medication prepared from Physarum extract
SO Fr. Demande, 14 pp.
CODEN: FRXXBL
AN 1979:556035 HCAPLUS
DN 91:156035

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	FR 2385730	A1	19781027	FR 1978-8962	19780328
	FR 2385730	B1	19810710		
	GB 1576891	A	19801015	GB 1977-12919	19770328
	NO 7801035	A	19780929	NO 1978-1035	19780322
	AU 7834479	A1	19790927	AU 1978-34479	19780323
	AU 519066	B2	19811105		
	ES 468236	A1	19781201	ES 1978-468236	19780327
	JP 54002310	A2	19790109	JP 1978-35269	19780327
	BE 865369	A1	19780928	BE 1978-186314	19780328
	FI 7800933	A	19780929	FI 1978-933	19780328
	DK 7801346	A	19780929	DK 1978-1346	19780328
	SE 7803456	A	19780929	SE 1978-3456	19780328
	NL 7803291	A	19781002	NL 1978-3291	19780328
	ZA 7801758	A	19790530	ZA 1978-1758	19780328
	AT 7802159	A	19810115	AT 1978-2159	19780328
	AT 363589	B	19810810		
	CA 1107646	A1	19810825	CA 1978-299788	19780328

L239 ANSWER 71 OF 73 CABA COPYRIGHT 2003 CABI
TI Mycolase, a new kind of systemic antimycotic.
SO Nature, UK, (1978) Vol. 273, No. 5659, pp. 235-236. 2 tab. See RMVM 12, 1222. 17 ref.
AU Davies, D. A. L.; Pope, A. M. S.
AN 78:65953 CABA

L239 ANSWER 72 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 20
TI THE RESPONSE OF ETHYLENE TREATED TOMATO PLANTS TO INFECTION BY VERTICILLIUM-ALBO-ATRUM.
SO PHYSIOL PLANT PATHOL, (1976 (RECD 1977)) 9 (3), 215-226.
CODEN: PPPYBC. ISSN: 0048-4059.
AU PEGG G F
AN 1977:164524 BIOSIS

L239 ANSWER 73 OF 73 HCAPLUS COPYRIGHT 2003 ACS
TI Enzymic degradation of septa in hyphal wall preparations from a monokaryon and a dikaryon of Schizophyllum commune
SO Journal of General Microbiology (1974), 83, Pt. 2, 359-68
CODEN: JGMIAN; ISSN: 0022-1287
AU Wessels, J. G. H.; Marchant, R.
AN 1974:548174 HCAPLUS
DN 81:148174

=> d ab 1,5,7,12,20,23,24,41,42

L239 ANSWER 1 OF 73 CABA COPYRIGHT 2003 CABI

L239 ANSWER 5 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB Production of **transgenic** plants with fungus disease-resistance is discussed, with respect to: race-cultivar-specific resistance traits (e.g. the Hm locus in maize (Zea mays) conferring resistance to Helminthosporium carbonum race 1); a two-component system for non-specific resistance (e.g. transfer of the Cladosporium fulvum avr9 gene into tomato (Lycopersicon esculentum) containing resistance gene Cf9

under a pathogen-inducible promoter); compounds toxic to fungi (e.g. cloning of a peanut (Arachis hypogaea) stilbene-synthase gene in tobacco (Nicotiana tabacum) for resistance to Botrytis cinerea); antifungal proteins (e.g. **chitinase** (EC-3.2.1.14) and endo-1,3-beta-D-**glucanase** (EC-3.2.1.39), ribosome inactivating protein and other antifungal proteins); and inhibitors of fungal enzymes (e.g. polygalacturonase-inhibitor). Increasing the knowledge of the molecular bases of pathogenicity and resistance should lead to development of new crop improvement strategies in future. (20 ref)

L239 ANSWER 7 OF 73 MEDLINE DUPLICATE 4
AB The Nicotiana tabacum ap24 gene encoding a protein with antifungal activity toward Phytophthora infestans has been characterized. Analysis of cDNA clones revealed that at least three ap24-like genes are induced in tobacco upon infection with tobacco mosaic virus. Amino acid sequencing of the purified protein showed that AP24 is synthesized as a preproprotein from which an amino-terminal signal peptide and a carboxyl-terminal propeptide (CTPP) are cleaved off during post-translational processing. The functional role of the CTPP was investigated by expressing chimeric genes encoding either wild-type AP24 or a mutant protein lacking the CTPP. Plants expressing the wild-type construct resulted in proteins properly sorted to the vacuole. In contrast, the proteins produced in plants expressing the mutant construct were secreted extracellularly, indicating that the CTPP is necessary for targeting of AP24 to the vacuoles. Similar results were obtained for vacuolar **chitinases** and beta-1,3-**glucanases** of tobacco. The extracellularly targeted mutant proteins were shown to have retained their biological activity. Together, these results suggest that within all vacuolar pathogenesis-related proteins the targeting information resides in a short carboxyl-terminal propeptide which is removed during or after transport to the plant vacuole.

L239 ANSWER 12 OF 73 CABA COPYRIGHT 2003 CABI
AB Two genes encoding proteins with antifungal activity, RIP from **barley** and a **chitinase** (ChiA) from the bacterium **Serratia marcescens**, were introduced into tobacco under the control of the wound-inducible promoter wun1 or the constitutive 35S promoter. When these plants were infected with Rhizoctonia solani, they grew nearly as fast as uninfected tobacco plants and showed reduced infection symptoms compared with control plants. The fungal resistance of these transgenic tobacco plants was stably inherited.

L239 ANSWER 20 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB The hydrolytic enzymes **chitinase** (EC-3.2.1.14), cellulase (EC-3.2.1.4) and cellobiohydrolase (EC-3.2.1.91) partially digest isolated cell walls of potential pathogens and inhibit fungal growth in vitro. The enzymes act **synergistically** to inhibit fungal growth. Constitutive overexpression of a bean **chitinase** in **transgenic** plants enhanced resistance against fungal pathogens. Constitutive co-expression of **chitinases**, cellulase and cellobiohydrolase may have the potential to strengthen plant resistance against phytopathogenic fungi. The potential of **chitinase**, cellulase and cellobiohydrolase co-expression in alfalfa (Medicago sativa) using an acidic **glucanase** cDNA clone isolated previously from an elicited alfalfa (Medicago sativa) cell suspension culture and a genomic clone of rice **chitinase** (RCH10) was investigated. The complexity of the **glucanase** gene family in alfalfa was also studied. (0 ref)

L239 ANSWER 23 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB Constitutive expression of genes encoding a **chitinase** (EC-3.2.1.4) ora ribosome inactivating protein in **transgenic** plants confers partial protection against fungal attack. The imminent cloning of disease-resistance genes and identification of genes that

affect symptom development will provide attractive new opportunities for enhancing crop protection. Strategies for enhancing crop resistance to microbial pathogens were discussed under the following headings: (A) manipulation of single gene defense mechanisms - **chitinases** and **glucanases**, ribosome inactivating proteins, other single gene defense mechanisms and topical applications: (B) manipulation of multigenic defense mechanisms - enhanced stress metabolism, engineering new and modified phytoalexins; (C) manipulation of regulatory mechanisms - single perception and transduction, agrochemicals that induce resistance; (D) amelioration of symptoms; and (E) future prospects. Emerging novel strategies for engineering crop protection will be incorporated into ongoing breeding and integrated management efforts to match food supply with demand. (0 ref)

L239 ANSWER 24 OF 73 CABA COPYRIGHT 2003 CABI
AB Combinations of class I beta -1,3-**glucanase** and **chitinase**, which are induced in plants infected with microbial pathogens, are potent fungicides in vitro. This finding suggests that these enzymes may be important in the defence against fungal infection. To test this hypothesis and to identify other functions of these proteins, sense and antisense transformation assays of Nicotiana sylvestris with Ti vectors containing the coding sequence of **chitinase** gene Chn48 or beta -1,3-**glucanase** gene Gla from tobacco were carried out. Susceptibility of transformed plants to Cercospora nicotianae was not significantly different from that of non-transformed plants, indicating that the accumulation of the enzymes is not a limiting factor in the plant defence against this pathogen. Probable silencing of the **transgenes** by trans-inactivation at the mRNA level is suggested.

L239 ANSWER 41 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB A composition for controlling plant pathogens contains 1 or more cell membrane-degrading components (preferably a natural or synthetic lytic peptide which can penetrate, lyse or otherwise impair the pathogen's cell membrane) and 1 or more hydrolytic enzymes. The lytic peptide is preferably a mammalian defensin, cecropin, thionin, mellitin, or insect defensin, magainin, attacin, dipteris, sapecin, cacrutin or xenopsin. The enzyme is beta-1,3-**glucanase** and/or **chitinase** (EC-3.2.1.14). Also claimed is a **transgenic** plant carrying recombinant DNA sequences encoding 1 or more cell membrane-degrading components (as above) and 1 or more hydrolytic enzymes (as above). The **transgenic** plant is able to synthesize anti-pathogenically effective amounts of 1 or more of these components. Transformation is normally performed using a Agrobacterium tumefaciens binary vector system. Examples are provided of the stable transformation (by direct infection with DNA, or co-cultivation of plant tissue with A. tumefaciens, or protoplast treatment using PEG or electroporation) and regeneration of **transgenic** tobacco, carrot, sunflower, tomato, cotton, maize, and orchardgrass. (35pp)

L239 ANSWER 42 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB Plants which exhibit relative overexpression of intracellular **chitinase** (EC-3.2.1.14) and/or beta-1,3-**glucanase** genes are new. The overexpression is a result of genetic manipulation of the plants (or their ancestors). Also claimed are: recombinant DNA (I) and (II) for overexpression of **chitinase** and beta-1,3-**glucanase** genes, respectively, including a gene encoding 1 of the enzymes, under control of a promoter and operably linked to a terminator, and a gene encoding a selectable marker or screenable trait; a cloning or transformation vector containing (I) or (II); plasmid pMOG200, plasmid pMOG212, plasmid pMOG289, plasmid pMOG512 and their derivatives; bacteria harboring a plasmid; and a process for obtaining fungus-resistant plants by transformation and plant regeneration. The enzymes provide resistance to many phytopathogenic fungi. Their genes are modified by creating stop codons at their 3' ends, resulting in deletion of 3-10 C-terminal amino

acids from the **chitinase**, and 3-25 residues from the beta-1,3-**glucanase**. The genes are preferably under the control of the cauliflower-mosaic virus 35S promoter and are targeted to the apoplast of the plant. (55pp)

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L239 ANSWER 12 OF 73 CABA COPYRIGHT 2003 CABI
TI Expression of a ribosome inhibiting protein (RIP) or a bacterial chitinase leads to fungal resistance in transgenic plants.
SO Mechanisms of plant defense responses, (1993) pp. 446-448. Proceedings of the 2nd International Conference of the European Foundation for Plant Pathology, Strasbourg, France, 24-27 August 1992. 10 ref. Publisher: Kluwer Academic Publishers. ISBN: 0-7923-2154-5
AU Logemann, J.; Jach, G.; Logemann, S.; Leah, R.; Wolf, G.; Mundy, J.; Oppenheim, A.; Chet, I.; Schell, J.; Fritig, B. [EDITOR]; Legrand, M. [EDITOR]
AN 94:46413 CABA

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TI Expression of hydrolases in transgenic alfalfa plants; chitinase, cellulase and cellobiohydrolase gene expression in transgenic plant for fungus disease-resistance and crop improvement (conference abstract)
SO Plant Physiol.; (1993) 102, 1, Suppl., 167 CODEN: PLPHAY
AU Masoud S; Lamb C J; Dixon R A
AN 1993-09330 BIOTECHDS

L239 ANSWER 23 OF 73 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Emerging strategies for enhancing crop resistance to microbial pathogens; engineering disease-resistance in transgenic plant for crop improvement (conference paper)
SO Curr.Plant Sci.Biotechnol.Agric.; (1993) 45-60 CODEN: 9999T
AU Lamb C J
AN 1993-14530 BIOTECHDS

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EMERGING STRATEGIES FOR ENHANCING CROP RESISTANCE TO MICROBIAL PATHOGENS

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CIBA-GEIGY[1], Sammel Roberts Noble Famdation[2].

There are marked differences in the pattern of host gene expression in incompatible plant: microbial pathogen interactions compared with compatible interactions,associated with the elaboration of inducible defenses. Constitutive expression of genes encoding a chitinase or a ribosome-inactivating protein in transgenic plants confers partial protection against fungal attack, and a large repertoire of such antimicrobial genes has been identified for further manipulation. In addition, strategies are emerging for the manipulation of multigenic defenses such as lignin deposition and synthesis of phytoalexin antibiotics by overexpression of genes encoding rate determining steps, modification of transcription factors or other regulatory genes, and engineering production of novel phytoalexins by interspecies transfer of biosynthetic genes. The imminent cloning of disease resistance genes, futher molecular dissection of stress signal perception and transduction mechanisms, and identification of genes that affect symptom development will provide attractive new opportunities for enhancing crop protection. Combinatorial integration of these novel strategies into ongoing breeding programs should make an important contribution to effective, durable field resistance.

MANIPULATION OF SINGLE GENE DEFENSE MECHANISMS

Chitinases and glucanases. One strategy is to express in a constitutive manner defense genes that are normally only induced as a result of pathogen attack. The most attractive initial candidates for this approach are genes encoding chitinases and ß1,3 glucanases. Thus some of these lytic enzymes have been shown to be active *in vitro* against various pathogens, and since the active antimicrobial agents are individually encoded by single genes, these defense systems should be highly amenable to manipulation by gene transfer. The first report of success with this approach was the expression of a bean vacuolar chitinase gene under the control of the strong constitutive promoter of the cauliflower mosaic virus(CaMV) 35S transcript in tobacco and *Brassica napus* which resulted in decreased symptom formation by *Rhizoctonia solani* the causative agent of post-emergent damping-off. Significant reduction in fungal growth and delay in disease

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development were observed and in the case of *B.napus* protection approached potentially useful levels with respect to reduced crop damage at inoculum densities likely to be encountered in the field. It is not clear whether the protection afforded by the chitinase transgene reflects a direct lytic effect killing the pathogen on contact or perturbation of the growth of the pathogen, slowing it with respect to the activation of the endog enous pathogen inducible defenses.

High level constitutive expression of the chitinase transgene in tobacco was obtained in these studies suggesting that a key aspect of the protection may be the engineered deployment of an effective defense mechanism prior to the activation of natural inducible defenses. However, since this chitinase gene was transferred from bean, it cannot be excluded by that the properties of chitinase encoded the transgene are different from those of the endogenous tobacco chitinases, e.g.substrate specificity V_{max} K_m stability and that confrontation of the pathogen with this unfamiliar chitinase rather than its manner of expression was critical for effective protection. Interestingly, transfer of a tobacco basic vacuolar chitinase gene under the control of the CaMV 35S promoter into the closely related species *Nicotiana sylvestris* did not give effective protection against cereaspore nicotianae,even in transgenic plants exhibiting consititutively high levels of chitinase activity.

Glucanases have also been expressed in plants , but no phenotypic effects have been reported to-date. However, glucanases and chitinases can act synergistically against fungi *in vitro* as might be expected from consideration of the organization of the cell wall at the mycelial tip. Hence, plants that constitutively express both chitinase and glucanase may exhibit greater disease control than plants expressing either one alone and experiments are in progress to test this hypothesis. Since plants express a variety of different chitinases and glucanases, there is a large potential repertoire of lytic enzyme genes, and optimal protection in the field may require manipulation of the expression of appropriate combinations of several genes encoding lytic enzymes with complementary activities.

Ribosome-inactivating proteins. Ribosome-inactivating proteins (RIP) such as the barley seed RIP.its wheat homolog tritin and the related ricin A-chain inhibit protein synthesis by specific RNA N-glycosidase modification of 28S rRNA. RIPs do not inactivate self ribosomes but show activity towards ribosomes from distantly related species including fungi, and purified barley RIP inhibits the growth of fungi *in vitro*. Expression of the barley RIP cDNA under the control of a wound-inducible promoter in transgenic tobacco plants confers protection against the soil-borne pathogen *Rhizoctorma salani* as judged by height differences between control and transgenic plants grown in infected soil, although direct measurements of the effect of the transgene on lesion size and fungal growth were not reported.

The successful use of RIPs in crop protection will depend on the extent to which their cytotoxicity is detrimental to the host cells. although introduction of the barley RIP cDNA under control of a wound-inducible promoter that is also strongly active in pollen and floral organs did not cause infertility in the primary transformants and tobacco plants expressing the cDNA under the control of the CaMV 35S promoter apparently grow normaly. Moreover. RIPs from differnet species vary considerably in their inhibitory specificities, and the host cytotoxicity of otherwise potentially useful RIPs may be alleviated by targeting to the extracellular space or vacuole and inducible expression. RIPs interact synergistically with chitinases *in vitro* suggesting that uptake into the fungus may be a limiting factor such that digestion of the mycelial cell wall increase effective antimicrobial activity.

Other single gene defense mechanisms. While random screening of plant extracts for antimicrobial proteins may provide potential leads for novel antimicrobial genes several targeted strategies appear particularly worthwhile. For example, stress inducible proteins in addition to specific chitinases and glucanases likely have antimicrobial properties, and other PR protein genes with cryptic functions have been manipulated to give strong consititutive expression. Constitutive expression of the tobacco PR-1 gene has no apparent effect on symptom development in transgenic tobacco inoculated with tobacco mosaic virus(TMV), but recent data indicate delaved onset of blue mold caused by *Peronospora tabacina*. The tobacco PR.5 gene has also been constitutively expressed in transgenic tobacco. No effect on symptom formation by TMV was observed. However, since PR.5 is closely related to osmotins with *in vitro* antifungal activity, this class of PR protein genes are clearly candidates for more extensive pathogen testing in transgenic plants engineered for over-expression. While these studies involve manipulated expression of specific PR protein genes in the homologous plant interspecies gene transfer may be particularly effective since this strategy allows the introduction of proteins with potentially distinctive properties compared to the resident battery of inducible antimicrobial activities, in addition to engineering constitutive expression.

Floral organs contain high levels of glucanase, chitinase and other PR proteins, which may reflect anticipatory expression of specific defense mechanisms in particularly vulnerable tissues, although non-defense functions for a glucanase abundant in stigma have been proposed, and a specific extracellular chitinase is able to rescue a carrot mutant defective in somatic embryo genesis. Irrespective of whether such enzymes have a primary function in development or defense the expression of specific PR protein genes in floral organs provides a rich source of potential antimicrobial proteins with possibly novel properties. Likewise seeds appear to be an excellent source of antimicrobial proteins. For example in addition to chitinases, glucanases and

RIPs specific lectins often accumulate to high levels during embryogenesis, and a lectin from stinging nettle has been shown to have strong antimicrobial activity although its mode of action remains to be determined. Interestingly the corresponding gene encodes a polypeptide with an active chitinase at the C termimus separated from the two lectin domains by a short linker sequence.

The search for active antimicrobial genes has not been restricted to the genomes of higher plants. For example expression of a chitinase from the bacterium *Serratia marcescens* apparently confers protection against *Rhizoctoniz solani* and the genes encoding antimicrobial proteins involved in esatblished biological control systems have obvious potential for manipulation and like wise introduction of non-plant lysozymes.eg.phage. The cecropin family of antimicrobial peptides from the giant silk moth are attractive candidates for manpulation lysizyme or mammalian lysozymes. might enhance resistance to bacterial pathogens. Bovine lysozyme is active *in vitro* against a range of both gram positive bacte ria(eg. *Clavibacter*) and gram negative bacteria, including phytopathogenic *Agrobacterium*. *Erwinia*. *Pseudomonas* and *Xanthomonas* species and preliminary data indicate that constitutive expression of the bovine gene in tobacco gives some protection against *Pseudomonas syringae* pv. *tabaci*.

Topical applications. While it is generally envisioned that the delivery of antimicrobial proteins will be through the creation of transgenic plants with appropriate expression of the transgene(s), antimicrobial proteins with direct modes of action such as liytic enzymes could also be delivered by topical application. For example bovine lysozyme produced in *Pichea pastoralis* is highly stable and spraying tomato with lysozyme purified in one step from this yeast expression system protects against *P.syringae* . This approach avoids the time and expense of producing transgenic plants. and hence may be particularly attractive for annual crops that are difficult to transform e.g.cereals, crops where extensive crossing of the transgene into breeding lines is required, and perennial plantation crops, where the agronomic impact of transgenic germplasm takes many years to be realized. Moreover, since antimicrobial proteins appear to act syneigistically topical application would allow flexible deployment of different combinatorial formulations individually tailored for specific problems. The costs of protein production and application will be critical factors in determining the usefulness of this approach although lysozyme treatment of seed stocks contaminatd with bacterial pathogens e.g. *Cornybacterium michiganese* pv *michiganese* in hybrid tomato seed and *Xanthomonas campestris* pv *carotae* in hybrid carrot seed is immediately attractive given the targeted mode of application and the lack of satisfactory altermatives.

MANIPULATION OF MULTIGENIC DEFENSE MECHANILSM

Enhanced stress metabolism. Defense responses such as phytoalexin biosyntheisis or lignin deposition in the cell wall

require the action of many genes. Prospects for enhancing the expression of such multigenic defenses depend on either the identification of a rate determining step, manipulation of which impacts flux through the multistep pathway leading to the elaboration of the protective agent or the identification of regulatory genes that condition the coordinate expression of batteries of functionally interdependent defense genes.

Phenylalanine ammonia lyase (PAL) catalyzes the first reaction in the synthesis of a wide range of natural products based on the phenylpropane skeleton including lignin monomers as well as certain classes of phytoalexins (eg.pyterocarpans in Leguminouseae and furanocoumarins in Solanaceae and Umbelliferae). Analysis of a series of transgenic tobacco plants in which PAL activity was reduced to varying degrees following introduction of heterologous(bean) PAL sequences revealed a direct relationship between the level of PAL activity and accumulation of phenylpropanoid products indicating that PAL is a major rate determining step in this complex biosynthetic pathway. Moreover transgenic plants with suppressed phenylpropanoid biosynthesis show increased susceptibility to the tobacco pathogen *Cercospora nicotianae*. These studies show that it is possible to alter flux through a multistep metabolic pathway and hence impact the biological activity of the pathway prodcuts by manipulation of the expression of a single gene encoding a key regulatory enzyme and it will be of considerable interest to detemine over what interval plants exhibiting higher than wild type levels of PAL activity show corresponding increases in phenylpropanoid accumulation and pathogen resistance.

It may also be possible to manipulate flux into specific branch pathways of phenlpropanoid biosynthesis. Thus a comparison of induced enzyme activities in cell suspension cultures of two chickpea lines one resistan and one susceptible to the devastating fungal pathogen *Ascochyta rabiei* indicates that the massive increase in pterocarpan phytoalexin production in the resistant cultivar compared to the susceptible cultivar is primarily regulated by differential induction of isoflavone 2-hydroxylase activity is a conceptually simple strategy for increasing phytoalexin accumulation in this particular case although the relatively large constitutive pool of isoflavonoid precursors in chick peasuggests that it would be important to avoid leaky expression of the transgene during normal development in the absence of an applied stress.

Anionic peroxidases in the cell wall catayze the production of phenolic radicals for the oxidative polymerization of lignin from cinnamyl alcohols and may also be involved in other oxidative reactions at the cell surface implicated in plant defense such as the cross-linking of cell wall structural proteins. In tomato there is a marked induction of two linked genes encoding highly anionic peroxidases (TAP1,2) in an incompatible interaction with an avirulent form of *Verticillium albo-atrum*,with only weak induction in the compatible interaction

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with a virulent form of this vascular pathogen. Expression of one of these genes in transgenic tobacco under the control of either its own promoter or the CaMV 35S promoter resulted in a massive increase in anionic peroxidase activity, and these plants apparently showed 3 substantial increase in resistance to *Peromospora parasitica* as measured by symptom development and fungal sporulation.

These emerging studies indicate that it should be possible to enhance the effectiveness of multigenic defense mechanisms by the identification and manipulation of genes encoding key rate determining steps differentrally regulated in incompatible versus compatible interetions. While various aspects of phenylpropanoid stress metabolism are the most amenable to this approach at present our understanding of the biosynthesis of the antimicrobial benzophenanthridine alkaoids is rapidly increasing, and a key branch-point enzyme in this pathway has recently been cloned. Manipulation of alkaloid biosynthetic pathways for the production of both plant protective compounds and pharmacologically active compounds will likely become a major area of transgenic research in the near future. Preliminary attempts have been made to increase indole alkaloid biosynthesis in transgenic tobacco by overxpression of the enzyme trytophan decarboxylase which catalyzes the first committed step.

Considerable efforts have been devoted to the characterization of defense gene promoters and the identification of specific *cis*-elements involved in activation by pathogen attack. A number of promoter: reporter gene fusions have been shown to be induced by fungi, bacteria, viruses, insects and treatment with varios elicitors salicylic acid ethylene and woudning. Many stress-inducible promoters also show developmental expression apparently in the absence of applied stress eg. the promoters of PAL and CHS genes are active in root tips and certain floral organs as well as being activated by several biological stress signals. However an anionic peroxidase (TAP.1) promoter from tomato appears to be exclusively stress inducible, and such promoters or synthetic promoters engineered from complex defense gene promoters to retain inducible expression in the absence of developmental expression may be very useful for delivering conditional targeted expression of antimicrobial proteins such as RIPs. Identification of promoters that are strongly activated as early as possible in a compatible interaction would be particularly useful since such promoters would allow the engineered expression of antimicrobial genes independent of molecular recognition of pathogen attack and in precisely those situations where the endogenous resistance mechanisms are not effectively deployed.

Functional analysis in protoplasts and transgenic plants has identified specfic promoter sequences involved in stress activation. For example a 125 bp region of the parsley PR protein 2 gene promoter is sufficient for induction in elicitor-treated protoplasts and also confers elicitor regulation on a CaMV 35S

minimal promoter. Functional analysis and studies of elicitor inducted changes in chromatin structure have identified the sequence CCTACC designated the H-box or related AC rich sequences, as important for the activation of PAL and CHS transcription by elicitors or during the hypersensitive response two H-box factors that have been purified to homogeneity from bean cells and this provides the basis for isolation and manipulation of genes involved in the terminal stages of a signal pathway for activation of inducible defenses. The H-box appears to operate in combination with a ciselement with a CACGTG core sequence designated the G-box for both developmental regulation and stress induction of the bean CHS15 gene. The G-box is part of a family of palindromic ciselements involved in diverse modes of gene expression including induction by wounding light. UV and abscisic acid and several G-box finding factors are members of the bZIP class of transcription factors. While modifications of combinatorial interactions between different ciselements may engineer promoters with novel properties for the regulation of individual antimicrobial genes corresponding modifications of the structure or regulation of specific *trans*-factors eg. G-and H-box *trans*-factor combinations will allow concerted manipulation of the expression of batteries of functionally related defense genes.

Engineering new and modified phytoaleixins. Since different plant species produce different phytoalexins. the interspecies transfer of biosynthetic genes provides the basis for engineering novel classes of phytoalexins in crop plants. Pathogens are often less tolerant of the phytoalexins of non-host species and the virulence of *Nectria hematococca* and possibly a number of other relatively unspecialized pathogens is dependent on their ability to detoxify host phytoalexins. Therefore confrontation of such pathogens with unfamiliar phytoalexins is potentially an attractive strategy for enhancing resistance (Fig.1).

The phytoalexin resveratrol is synthesized by stilbene synthase in a single step from p-coumaroyl-CoA and malonyl-CoA the same substrates used by chalcone synthase for production of isoflavonoid phytoalexin precursors in soybean bean and pea. The stilbene synthase gene from peanut has been introduced into tobacco such that resveratrol is produced on exposure to UV light or fungal elicitor but the effect of production of this new phytoalexin in tobacco in augmenting natural resistance afforded by the endogenous sesquiterpenoid phytoalesins and other inducible defenses has not set been reported.

A second example of the generation of a phytoalexin in one step from a common metabolite is the synthesis of the cyclic diterpene casbene from the isoprenoid precursor geranylgeranyl pyrophosphate. Casbene synthase has been cloned from castor bean and is an attractive target for transfer into unrelated species with distinct phytoalexins. Similarly, sesquiterpene cyclases catalyze the conversion of farnesyl pyrophosphate to the precursors of the sesquiterpenoid phytoalexins of the Solanaceae

and expression of a fungal sesquiterpene cyclase gene results in the accumulation of new sesquiterpenoids in transgenic tobacco including trichodiene the precursor of the trichothecene family of toxic natural products. However, transfer of genes for modification of the initial cyclization product would be required to produce antimicrobial sesquiterpenoids in non-solanaceous plants.

Interesting opportunities also exist for the modification of the natural phytoalexins of a plant species to render them more toxic to its pathogens. For example pisatin exisis as the (+) (6aS 11aS) stereoisomer in peas as does the related pterocarpan maackiain and the pea pathogen *N.hematococca* is much less sensitive to pterocarpan phytoalexins of the 6aS 11aS configuration than to those of the opposite stereochemistry reflecting the specificity of the substrate-inducible fungal cytochrome P450 enzyme pisatin demethylase for detoxification of (+) rather than (-) pterocarpans. Other legumes such as soybean and alfalfa produce paterocarpan phytoalexins (glyceollin and medicarpin respectively) of tho 6aR 11aR configuration and the stereochemistry of pterocarpans is believed to be determined by isoflavone reductase and pterocarpan synthase which catalyze the reduction of isoflavone to isoflavvnone and the final stereospecific ring closure respectively. Transfer of genes encoding these enzymes from species with(+) pterocarpan phytoalexins (eg.peanut and the leguminous tree *Sophora japonicum*) to species with(-) pterocarpan phytoalexins (eg.alfalfa and chickpea). or *vice versa* should result in the formation of stereoisomers that are less readily degraded by the pathogens of the recipient host plant. As a first step isoflavone reductases have been purified and cloned from alfalfa pea and chickpea.

Other possible strategies for enhancing the effective ness of endogenous phytoalexins have emerged from studies of relationships between structure and antinicorbial activity. For example lipophilicity is an important feature if isoflavonoid phytoalexins such that the nonprenylate precursors of antimicrobial prenylated isoflavonoids such as wighteone and kievitone lack activity and virulence of *Fusarium solani* is associated with the ability to detoxify kievitone by hydration of the prenyl moiety. Moreover prenylated isoflavones have stronger antifeeding activity against Coleoptoran insects than non-prenylated precursors suggesting the potential for engineering protection against both insects and fungi by manipulation of prenytransferase genes. A labile dimethylallyl pyrophosphate:3,9-dihydroxypterocarpan 10-dimethylallyl transferase which prenylates the precursor of the bean phytoalexin phaseollin and can also prenylate the alfalfa phytoalexin medicarpin has been purified from elicited bean cells and an elicitor inducible cytochrome p450 for cyclization of the prenyl side chain as found in phaseollin and the soybean phytoalexin glyceollin has been characterized in soybean. In addition to prenylation methylation

can also have a major impact on antimicrobial activity as implied by the mechanism for pisatin detoxification in *N.hematococca*. A number of methyltransferases for phytoalexin intermediates have recently been isolated as a first step toward manipulation in transgenic plants.

MANIPULATION OF REGULATORY MECHANISMS

Signal perception and transduction. Many of the well characterized didease resistance genes appear to provide early recognition of a subset of the total races within a pathogen species often closely correlated with hypersensitive necrosis and defense activation. Resistance genes and the corresponding avirulence genes are dominant or partially dominant and a number of resistance genes often exhibit a high degree of intrinsic instability. For example unidirectional conversion of resistant alleles of the maize *Rpl* locus. Which conditions resistance to *Puccinia sorghi* to the recessive susceptible state is quantitatively allele-specific and occurs with a frequency of 10^{-2} to 10^{-4}. This instability may reflect unequal recombination within complex loci which could provide a potential mechanism for the generation of new recognitional specificities. Major resistance genes are often not durable in the field because of pathogen mutation although in some cases resistance remains unbroken after 20-30 years field use and in at least one case such durability reflects constrains on the pathogen arising from a role for the corresponding avirulence gene in fitness.

Strategies for cloning resistance genes include:differential expression with respect to genotype tissue specificity or physiological conditions; transposon tagging map based cloning biochemical characterzation of binding sites for race specific elicitors and shotgun cloning by function. This last approach may now be feasible because of the development of balllistic delivery systems for transient expression in plant tissues and the characterization of a low-molecular weight glycolipid race specific elicitor conditioned by the *P.syringae avrD* gene. This allows screening of pools of soybean cDNAs from the genotype containing the dominant allele of the correeponding resistance gene in bombarded tissue of a genotype containing recessive alleles. The assay is based on the appearance of a local hypersensitive response to the race specific elicitor against a background of non-responding tissue. Moreover, the recent characterization of this glycolipid race specific bacterial elicitor and a peptide race specific fungal elicitor encoded by the *Cladosporium fulvum Avr9* gene now allows for direct biochemical study of the binding to putative products of the corresponding resistance genes in soybean (*Rpg4*) and tomato(*Cf9*) respectively. Efforts to clone resistance genes have also been facilitated by emerging transposon tagging and genome mapping tools in model systems such as tomato and *Arabidopsis* and in the latter case by the characterization of pathosystems that exhibit gene-for-gene specificity. For example the *Arabidopsis RPM1* locus. Which establishes incompatibility to *P.syringae* pv

maculicola carrying the corresponding avirulence gene maps to the top of chromosome 3 and has been located on a 270 kb YAC clone. Likewise the tomato 12 locus for resistance to *Fusarium oxysporum* f.sp *hyropersise* race 2 appears to be located within 40kb of an RFLP marker and the isolation of several resistance genes from such model systems appears imminent.

In addition to providing insights into the molecular mechanisms underlying the perception of specific pathogen racee and the initial stages of signal transduction for induction of hypersensitive necrosis and defense activation the molecular cloning of resistance genes will allow the enhancement of surveillance mechanisms by the pytamiding of allelic combinations of resistance genes not readily accomplished by conventional breeding. This strategy should reduce the frequency of pathogen mutation to virulence by avoidance of recognition by the host and as structure:activity relationships emerge new recognitional specificities might be engineered by domain swapping and related modifications. Morcover gene transfer between pathovars has revealed the presence of avirulence genes not pathogenic on the host plant eg. *avrD* was isolated from *P.syrogae* pv *tomato* but its function and the presence of the corres ponding resistance gene in soybean was only revealed after mobilization into *P.syringae* pv *glycinea* because the tomato pathovar is not pathogenic on sovbean. These studies suggest that interspecies transfer of resistance genes may allow the introduction of novel specificities into crop species and that non-host resistance as well as race-cultivar specificity may in some instances be conditioned by gene-for-gene interactions. Hence transfer into tomato of the soybean resistance gene corresponding to *avrD* might protect against *P.syringae* pv *tomato*.

The molecular basis of the perception of pathogen attack is also being revealed by biochemical approaches. Thus a specific 3.3 6-linked glucan heptanner has been identified as the minimal active fragment of has been identified as the minimal active fragment of an elicitor isolated from the mycelial cell walls of the soybean fungal pathogen *Phytophthora megasperma* f.sp.*glycinea*. This elicitor does not appear to be confined to specific races of the pathogen, or active with only specific cultivars of the host, and hence may be involved in non-host resistance although race-cultivar specificity might reside in the release of the active fragments from the mycelial wall or their subsequent breakdown rather than in the structure of the elicitor *per se*. A 70kD plasma membrane protein that exhibits high affinity binding of elicitor active heptaglucoside isomers (K_d=3nM) has been purified as a putative receptor and represents an attractive target for molecular cloning. *Phytophthora cryptogea* and *p.capsici* two pathogens causing systemic leaf necrosis on tobacco produce closely related 98-amino acid petides cryptogein and capsicein respectively which are sufficient to cause necrosis and application of these peptides protect tobacco against *P.nicotianae* which does not produce such an elicitor.

Cryptogein causes visible necrosis at a dose of 1 ug per plant, whereas 50 times as much capsicein is required to produce a similar reaction. The latter induces protection in the near absence of necrosis, suggesging that the protective and necrosis-inducing activities of these peptides may be distinct . Appropriate expression of capsicein and related sequences in transgenic plants may provide a new approach to engineering protection and the putative plant receptors for these host range determinants are also attractive targets for future manipulation. The recent discoveries of the peptide systemin and salicylic acid as signal molecules in the systemic induction of defenses in response to wounding and hypersensitive necrosis respectively like wise provide the basis for the characterization and manipulation of receptors involved in stress signaling (Fig.2). A soluble binding site for salicylic and that appears as an aggregate of approximately 650 kD has recently been reported in tobacco.

There is considerable interest in the signaling mechanisms between recognitional events at the cell surface such as elicitor binding to plasma membrane receptors and the activation of inducible defenses Ca^{2+} appears to be essential for the induction of phytoalexin biosynthetic genes in response to elicitors and influx of Ca^{2+} leading to increased cytosolic levels has been observed in the early stages of the response . H^+ influx and K^+ efflux predede the Ca^{2+} response and experiments with polyene antibious suggest that these ion fluxes may likewise be causally related to defense activation . Elicitors also cause rapid changes in the phosphorylation of specific plasma membrane and nuclear proteins and protein kinase inhibitors block ethylene and PAL induction. However, while these physiological experments disclose possible early events they do not reveal the specific molecular machinery involved in signal transduction. Such insights necessary for biotechnological manipulation will likely come from genetic dissection of signal transduction and step wise biochemical analysis of the pathways either forward from signal perception or backward from defense gene transcription factors.

Hypersensitive necrosis is associated with an oxidative burst leading to the production of activated oxygen species such as superoxide and H_2O_2. Elicitor induces the transient accumulation of H_2O_2 at the surface of soybean cells with 2 to 3 minutes and simultaneous addition of catalase or ascorbate blocks phytoalexin accumulation. Hence the oxidative burst may play a role not only in the rapid cross-linking of cell wall structural proteins but also in signaling the nucleus for defense gene activation. The stress activation of oxidative metabolism at the plant cell surface is strikingly similar to macrophage activation in the immune system and fertilization induced cross linking of extracellular matrix proteins in *xenopus* eggs. Linkewise the transcription factor NFKB is regulated by H_2O_2 and it will be of interest to determine whether the underlying molecular machinery involved in the plant oxidative burst protein cross linking and

defense gene regulation resembles the animal systems.

Agrichemicals that induce resistance. Several classes of compounds have the potential to act as inducers of natural resistance mechaniams in agricultural settings and chemicals with such indirect modes of action may offer attractive alternatives or additions to existing contact fungicides in integrated pest management. For example probenazole the acitve ingredient in Oryzemate (Meiji Seika) is widely used in the Pacific rim for control of rice blast caused by *Magnaportha grisea*. Probenazole and its metabolites do not themselves have antifungal activity but the fungicide evokes significant alterations in host physiology which may contribute to protein including the production of fatty acids with antifungal activity eg. a-linolenic acid, and increases in the activities of lignin biosynthetic enzymes eg. PAL catechol o-methyltransferase and peroxidase.

Other synthetic inducers of protection not currently available commercially are 2.6-dichloroisonicotinic acid (INA)(Fig.2) and N-phenylsulforyl-2-chloroisonicotin amide (PBSI).INA has no direct effect on bacterial or fungal pathogens but induces the same set of defense genes that are systemically activated by local pathogen infection and is also efficacious in *A.thaliana* against *Peronospora parasitica* and *Pseudomunas syringne* inducing genes related to the PR.1 PR.2 and PR.5 genes of tobacco. PBSI has no effect on penetration of *M.grisea* spores through cellophane *in vitro* but like probenazole significantly protects rice against blast. Only preliminary sutdies have been performed on changes in metabolism and gene expression that correlate with protection.

Recont advance in our understanding of plant defense mechanisms provide opportunnes for the development of novel chemistries which mimic natural inducers of plant defense responses eg. salicylic acid fatty acids jasmonic acid and systemin (Fig.2) as well as the use of transgenic plants containing defense gene promoter-reporter gene fusions as screening tools.

AMELIORATION OF SYMPTOMS

Crop damage and loss of productivity reflect the effects of disease symptoms and hence may be prevented by the amelioration of these symptoms as well as by the direct antibiotic inhibition of microbial growth. In many cases symptoms result from the action of microbial toxins some of which are highly host specific. For example production of phaseolotoxin by *Pseudomonas-syringne* pv *phaseolicola* is a key component in the generation of halo blight disease symptoms infected bean or tobacco plants. This tripeptide acts by inhibition of ornithine carbamoyl transferase. and expression in transgenic tobacco of a gene from the pathogen which encodes a phaseolotoxin insensitive form of the enzyme reduces symptom severity. Similarly expression of a gene conferring resistance to tabtoxin in transgenic tobacco reduces both toxin sensity and disease development, and a recent

report has identified a specific reductase in maize that is responsible for resistance to *Cochliobolus(Helminthosporium)carbonum* race 1 by detoxification of the HC-toxin.

Manipulation of plant inhibitors of fungal polygalacturonases may provide a second avenue for diminshing the impact of disease. Thus fungal endo a-1. 4D-polygalacturonases catalyze the fragmentation and solubilization of plant cell wall homo-a-1 4D galacturonans thereby facilitating colonization and nutrient release. All dicots examined contain cell wall associated specific inhibitors of fungal endopolygalacturonases (PGIP) which have no effect on plant or bacterial endopoygalacturonase or other microbial pectic enzymes. The recent cloning of a bean PGIP gene provides the basis for overexpression to inhibit fungal colonization. Increased levels of PGIP should also reduce the turnover of oligogalacturonide fragments released from the plant cell wall thereby increasing the potential effectiveness of these endogenous signals for defense activation.

Some component of disease development appear to involve the active participation of the host and this provides further opportunities for engineering reductions in symptom severity. For example ethylene production is stimulated in response to pathogen attack and may mediate plant stress responses. Interestingly and ethylene insensitive mutant of *A.thaliana ein* 2 shows reduced symptom development to infection by *P.syringae* pv *tomato*. Another ethylene insensitive mutant *ein1* does not show reduced symptoms and hence symptom development may not be a simple consequence of ethylene production. However, these data indicate that specific plant genes contribute to symptom development and hence the damaging effects of disease might be alleviated by gene transfer approaches.

FUTURE PROSPECTS

The recent studies of the effects of overexpression of genes encoding chitinase and other antimicrobial proteins are very encouraging but it seems likely that combinations of such genes will often be needed to engineer levels of protection that will be useful in the field. Such combinatorial deployment of antimicrobial genes each giving partial protection. may in fact be desirable since this should reduce the selection pressure on the pathogen and hence prevent the evolution of resistance or avoidance mechanisms such as minor modifications in cell wall structure or production of inhibitors to reduce the effectiveness of lytic enzymes. We can anticipate that the present repertoire of antimicrobial genes will be supplemented by the discovery of genes encoding novel antimicrobial activities from diverse sources as well as the manipulation of multigenic defenses and the underlying signal perception and transduction mechanisms. Phytopathogenic fungi are formidable adveraries and we must hope that these emerging novel strategies for engineering crop protection can be successfully incorporated into ongoing breeding and integrated management efforts to match food supply with

demand in the next century.

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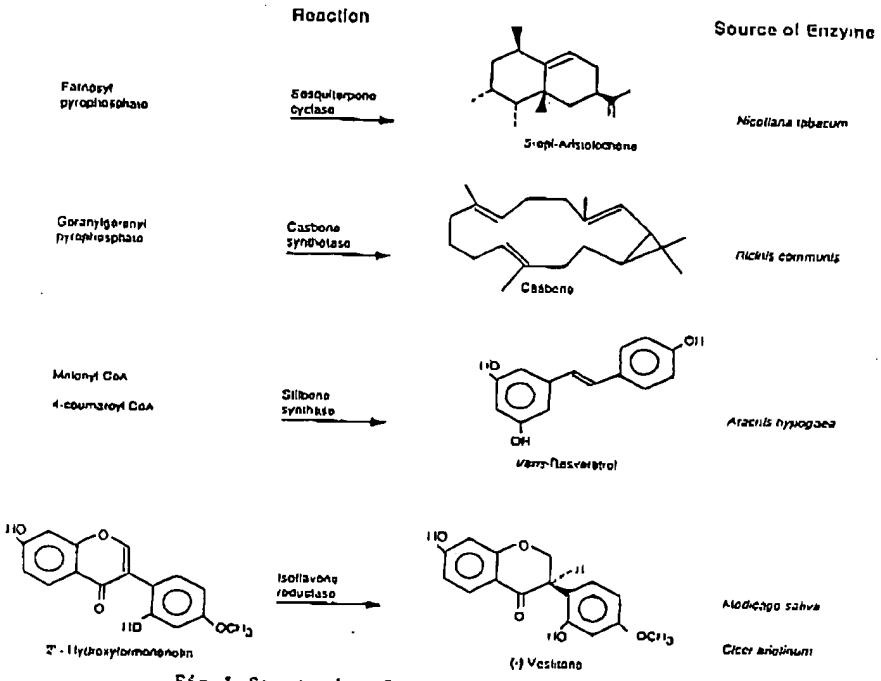
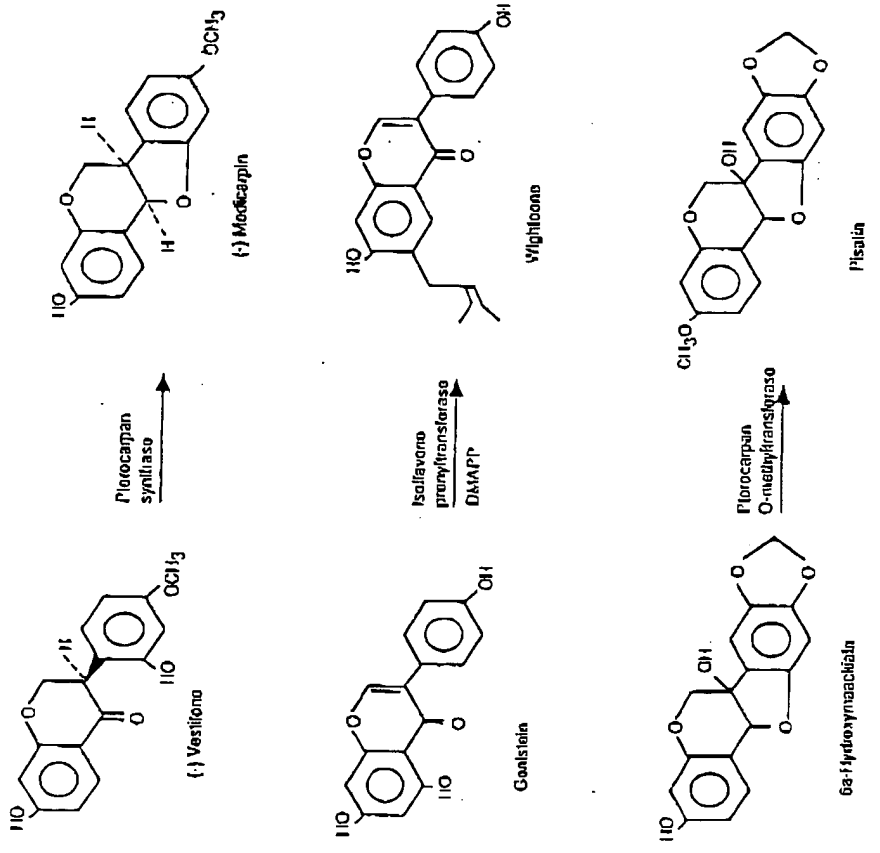


Fig.1 Strategies for engineering new or modified phytoalexins.

Fig 1. continued



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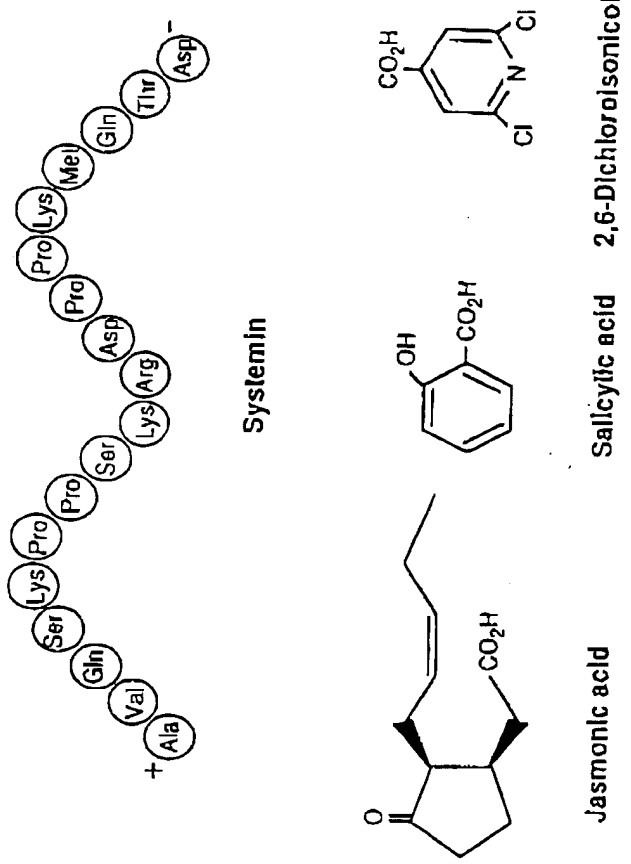

Fig.2 Stress signal molecules

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957

REDUCED WOUND INDUCTION OF DAHP SYNTHASE DUE TO EXPRESSION OF ANTISENSE DAHP SYNTHASE RNA IN POTATO

J.D. Jones [1], J.M. Henstrand [2], A.K. Handa [1], and S.C. Weller [1], [1]Horticulture Dept., Purdue University, W. Lafayette, IN 47907, [2] Institut für Pflanzenwissenschaften, Zurich, Switzerland

Transgenic potato plants containing an antisense RNA gene for a 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase have been produced in order to examine the roles of this enzyme in plant growth and development. The chimeric DNA constructs used for transformation contained the transcript start site and the first intron in both antisense and sense orientations under the control of the CaMV 35S promoter. Tubers from several independent transgenic potato plants were wounded and levels of DAHP synthase enzyme activity, mRNA, and antisense RNA were determined 0, 12, and 24 hours after wounding. Among the transgenic tubers expressing DAHP synthase antisense RNA, 4 showed a 40% reduction in wound-induced DAHP synthase enzyme activity and mRNA levels, while no such reduction was observed in transgenic potato tubers containing the chimeric sense construct. Transgenic antisense plants which showed a reduction in the wound-induced expression of DAHP synthase were also more sensitive to glyphosate. With one exception, antisense DAHP synthase plants had similar vegetative dry weights and tuber yields compared to untransformed plants. Levels of DAHP synthase activity, protein, and mRNA are being examined further in tubers and other organs of transgenic antisense potato plants to determine the effect of DAHP synthase antisense RNA expression on the expression of cloned potato DAHP synthases during plant development and in response to wounding.

958

TRANSFORMATION OF THE FORAGE GRASS CAUCASIAN BLUESTEM VIA BIOLISTIC BOMBARDMENT-MEDIATED DNA TRANSFER.

C.I. Franklin and T.N. Trieu, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

The forage grass caucasian bluestem (*Bothriochloa caucasica* L.) is well adapted to warm season and different soil conditions in the south central United States. Development of an efficient genetic transformation system is a prerequisite for future genetic manipulations of caucasian bluestem to improve its forage quality. In this study, we have optimized conditions for DNA delivery via biolistic bombardment using the Dupont PDS 1000 particle gun. Seed explants and callus cultures of caucasian bluestem were bombarded with tungsten or gold particles coated with the plasmid (pBARGUS) DNA. Transient GUS assays were performed 24-48 hours after the bombardment. The maximum number of cells expressing GUS was achieved with gold particles when 10-15 day old explants were placed approximately 5 cm from the stopping plate and a pressure setting of 1550 psi was used. Two weeks after the bombardment, the cultures were transferred to bialophos selection medium. Four to 6 weeks after the transfer, small clumps of embryogenic calli resistant to bialophos were produced. Attempts are now in progress to regenerate plants from this putatively transformed embryogenic callus.

959

EXPRESSION OF HYDROLASES IN TRANSGENIC ALFALFA PLANTS

Sameer Masoud[1], Christopher J. Lamb[2], & Richard A. Dixon[1] [1]The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, OK 73402; [2]The Salk Institute for Biological Studies, Plant Biology Laboratory, 10010 N. Torrey Pines Road, La Jolla, CA 92037

Stimulation of lytic enzymes is one of several active plant defenses against microbial attack. The hydrolytic enzymes chitinase and β-1,3-glucanase partially digest isolated cell walls of potential pathogens, and inhibit fungal growth *in vitro*. The enzymes act synergistically to inhibit fungal growth. Constitutive over-expression of a bean chitinase in transgenic plants has been shown to enhance resistance against fungal pathogens. Constitutive co-expression of both chitinases and glucanases may have the potential to further strengthen plant resistance against phytopathogenic fungi. We are investigating the potential of glucanase and chitinase co-expression in alfalfa, using an acidic glucanase cDNA clone isolated previously from an elicited alfalfa cell suspension culture, and a genomic clone of rice chitinase (RCH10). Analysis of the tentative transformed plants will be presented. We also present preliminary data on the complexity of the glucanase gene family in alfalfa.

960

EXPRESSION OF FUNGAL LIGNIN DEGRADING ENZYMES IN PLANTS

Dennis Mathews[1], Sandra Austin-Phillips[1], Monorama John[1], Jesse Will[1], Mark Shahan[1], Richard Amasino[2], Richard Burgess[1] [1]Biotechnology Center, [2]Biochemistry Dept., Univ. Wisconsin, Madison, WI 53706

The lignin degrading enzymes lignin peroxidase (LiP) and manganese peroxidase (MnP) from the white rot fungus *Phanerochaete chrysosporium* were incorporated into plant expression vectors under control of the constitutive Mac promoter. Tobacco and alfalfa plants were transformed using *Agrobacterium*. Transgenic tobacco plants expressed LiP mRNA but protein and enzyme activity were not detected. Tobacco plants transformed with MnP did result in detectable protein and enzyme activity. Accumulation and localization of MnP did not appear to be affected by substituting a plant ER signal sequence for the endogenous fungal signal sequence. No visible effect on growth or development was observed in tobacco plants expressing MnP.

961

ENZYMES OF LEUCINE SYNTHESIS IN SPINACH CHLOROPLASTS

Petra Hagelstein[1], Michael Klein[2], & Gernot Schultz[1] [1]Botan. Inst., Tierärztl. Hochschule, D-3000 Hannover 71; [2]Zentrum Biochemie, Medizinische Hochsch., D-3000 Hannover 61, FRG.

The synthesis of leucine in plants from 2-oxo-isovalerate (OIV) and acetyl-CoA (leucine branch) was shown to be compartmented in chloroplasts (P. Hagelstein & G. Schultz 1992). The involved enzymes are: α-isopropylmalate (IPM) synthase, IPM isomerase, β-IPM dehydrogenase and 2-oxo-isocaproate (OIC)-glutamate aminotransferase. α-IPM synthetase was studied in hypotonically treated, purified spinach chloroplasts. K_m values for OIV and acetyl-CoA, in μM, were: 75 and 5. The enzyme was completely inhibited by 20 μM L- (and D-)leucine indicating a feedback inhibition mechanism. Optimal activity in 50 mM NaP_i buffer pH 8.0 was achieved by adding 2 mM K^+ and 1 mM Mg^{2+} (as chlorides). Pilot tests indicated the presence of a $NADP^+$ β-IPM dehydrogenase. OIC-glutamate aminotransferase was studied in the ultrafiltrate (Millipore exclusion size 5 kDa) of stroma from purified spinach chloroplasts. K_m values for OIC and Glu were: 120 μM and 8 mM. The enzyme showed optimal activity at pH 9.0 by adding 30 mM K^+, and was strongly inhibited by 20 mM L-leucine and L-isoleucine but not L-valine.

962

INDUCTION OF LIMONENE SYNTHASE ACTIVITY IN GRAND FIR CALLI BY THE OMISSION OF GROWTH REGULATORS AND BY AUTOCLAVED FUNGAL EXTRACTS.

Efraim Lewinsohn, Eric Worden, & Rodney Croteau Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Monoterpenes are important defensive chemicals produced by conifers against wounding, herbivore and pathogen attacks. The biosynthesis of these volatile compounds is induced in grand fir (Abies grandis, Pinaceae) saplings as a response to stem wounding. This induction consists of the appearance of at least six distinct monoterpene synthases that catalyze the production of different cyclic monoterpene olefins from geranyl pyrophosphate. Limonene is the major olefin generated in cell-free extracts of non-wounded tissues, while (-)-α- and (-)-β-pinene, the major products generated in extracts from wounded grand fir stems. Callus tissues were obtained from de-barked stem pieces of grand fir saplings and cultured on a modified solid Murashige and Skoog medium supplemented with 2% sucrose, 5 ppm 2,4-D and 0.1 ppm BAP. Calli transferred to similar medium but devoid of growth regulators displayed increased (8-fold) levels of limonene synthase activity, as compared to non-transferred calli, and 2-fold higher activity levels than calli transferred to complete medium, on a protein basis. Maximum enzyme activity levels were observed 48 h after the transfer, followed by a decline in the extractable activity. Further injuring of the callus tissues did not affect enzyme activity levels. Additionally, calli transferred to complete medium containing 1 mg FW/ml of autoclaved Penicillium brevicompactum extracts, a fungus isolated from grand fir cut stems, displayed similar increases in limonene synthase activity.

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CONTENTS

EXPRESSION OF A RIBOSOME INHIBITING PROTEIN (RIP) OR A BACTERIAL CHITINASE LEADS TO FUNGAL RESISTANCE IN TRANSGENIC PLANTS.

J. LOGEMANN, G. JACH, S. LOGEMANN, R. LEAH[1], G. WOLF[2], J. MUNDY[1], A. OPPENHEIM[3], I. CHET[4] AND J. SCHELL.

Max-Planck-Institut f. Zuchtungsforschung, Carl von Linne Weg 10, D-5000 Cologne 30, Germany; 1Copenhagen; 2Gottingen; 3Jerusalem; 4Rehovot.

ABSTRACT. In order to protect plants against fungal attack, two genes (RIP, ChiA) encoding proteins with in vitro antifungal activity were expressed in transgenic tobacco plants.
1. A barley derived cDNA clone (RIP) encoding a ribosome inhibiting protein. RIP inhibits protein synthesis in fungi by specific RNA N-glycosidase modification of the 28S RNA.
2. A chitinase gene (ChiA) derived from the bacterium *Serratia marcescens* with the ability to destroy hyphal tips of growing fungi.
Plants expressing RIP-protein or ChiA-protein under the control of the wound-inducible promoter *wun1* or the constitutive 35S-promoter were infected with the plant pathogenic fungus *Rhizoctonia solani*.
Whereas the growth of control plants was drastically reduced because of root and stem disease, RIP- and ChiA-transgenic plants were growing nearly as fast as uninfected tobacco plants and showed reduced infection symptoms. The fungal resistance of these transgenic tobacco plants was stably inherited.

1. Introduction

The destruction of crop plants by fungal pathogens is a serious problem worldwide and annually leads to losses of about 15%.
In order to control fungal diseases, fungicides are used which are helpful in several (not all) cases but can also cause environmental problems. To prevent plants from being destroyed by fungal pathogens one alternative approach is to introduce a gene into the plant genome which encodes a protein with antifungal activity and therefore enables the plant to protect itself against fungal attack.
Several genes from either plants or microorganisms (bacteria and fungi) encoding proteins with in vitro antifungal activity have been analysed. However only in a few cases it could be demonstrated that the observed in vitro antifungal activity correlated with in vivo protection in transgenic plants.
Broglie et al. (1991) demonstrated that the expression of a bean chitinase in transgenic tobacco leads to an increased resistance against *Rhizoctonia solani*. A bacterial chitinase (*Serratia marcescens*) was expressed in transgenic tobacco plants which also turned out to be protected against *Rhizoctonia solani* attack (Jones et al., 1988; Dunsmuir et al., 1991).

B. Fritig and M. Legrand (eds.), Mechanisms of Plant Defense Responses, 446–448.

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2. Results and Discussion

2.1 RIP

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2.2 ChiA

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We demonstrated that the expression of either a barley derived ribosome inhibiting protein (RIP) or of a bacterial chitinase ChiA (which differs in sequence and biochemical properties from the chitinase used by Dunsmuir et al.,) leads to protection against fungal attack in transgenic tobacco plants (Logemann et al., 1992, Jach et al., 1992).

2. Results and Discussion

2.1 RIP

RIP is encoded by a cDNA clone derived from barley seeds. It inhibits protein synthesis in target cells by specific RNA N-glycosidase modification of 28S rRNA. This RIP does not inactivate self ribosomes, but is active against fungal ribosomes.
In vitro studies with purified RIP demonstrated that low concentrations of RIP are cytotoxic to several fungi when combined with chitinases (Leah et al., 1991). It has been suggested that the chitinase enables RIP to enter the fungal cells in order to efficiently inhibit fungal protein synthesis.
The expression of the RIP-gene under the control of the wound- and pathogen inducible promoter *wun1* (Logemann et al., 1989; Siebertz et al., 1989) led to an accumulation of RIP-protein in wounded leaves, stems, and roots of transgenic tobacco plants. In order to test fungal resistance of tobacco plants the basidiomycete *Rhizoctonia solani* was used. As a soil living fungus *Rhizoctonia* causes disease on roots, stems and leaves attached to the ground, which leads to reduced growth of the plant and in extreme cases to plant death.
Transgenic tobacco plants expressing RIP-protein, as well as control plants lacking RIP were infected with *Rhizoctonia solani*. While the growth of the control plants was drastically reduced due to root and stem disease, RIP-transgenic plants grew nearly as fast as uninfected tobacco plants and showed markedly reduced infection symptoms. Analysis of fungal resistance of transgenic RIP-plants in the next generation showed that the resistant phenotype was stably inherited (Logemann et al., 1992).
Further analysis of transgenic tobacco plants expressing the RIP gene under the control of the constitutive 35S-promoter revealed high levels of RIP protein in leaves, stems and roots (about 0,5 to 1% RIP of total leaf protein).
Preliminary data indicate that these plants exhibit a degree of tolerance to infection that is higher even than in the *wun1*-RIP transgenic plants.

2.2 ChiA

The bacterial chitinase gene ChiA was derived from a specially selected strain of the soil bacterium *Serratia marcescens*. Earlier experiments with 203 different bacterial strains demonstrated that this *Serratia* strain was the most efficient biocontrol agent for the fungal pathogen *Sclerotium rolfsii* (Ordentlich et al., 1988). The ability to control fungal infection is localized on a small 4,7 Kb fragment since sub-cloning of this fragment into E. Coli produced strains capable of controlling the fungus (Shapira et al., 1989).
Further analysis showed that a subfragment (1,8 Kb) encodes a chitinase (ChiA) which turned out to be a very potent inhibitor of the growth of several fungi including the agriculturally important fungus *Rhizoctonia solani*.
The ChiA gene was expressed under the control of the 35S-promoter in transgenic tobacco plants. ChiA-mRNA and ChiA-protein was detected in leaves, stems and roots of transgenic tobacco plants. Using a new technique with dye-labeled chitin it was demonstrated that the ChiA protein is active in transgenic plants.

In several independent experiments ChiA-expressing seedlings of T1 derived 35S-transgenic tobacco plants were grown on *Rhizoctonia solani* infested soil (0,2 g/liter soil) under constant environmental conditions (25° C. 85% rel. humidity).
After 15 days about 80% of the ChiA-expressing plants survived whereas only 20-30% of the untransformed or vector-transformed control plants were alive (Jach et al., 1992).
Meanwhile it could be demonstrated that RIP- or ChiA-protein can also be expressed in important crops such as potato plants. Studies to test the resistance of such plants against *Rhizoctonia solani* and other agriculturally important fungal pathogens are in progress.

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GENETIC TRANSF RMATI N F N.
ß-1,3-GLUCANASE ENES FROM

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The defense responses pathogens include the indu which, directly or indirectl against the invading pathog enzymes, e.g. chitinases an Different chitinase and purified from *Cercospora* potential antifungal activi bioassays using *Trichoderma* a basic chitinase (chitinas ß-1,3-glucanase showed good three enzymes have therefor In sugar beet all three e space, due to the lack of chains. *C. beticola* is a f the intercellular space. deposition of the antifung cellular space.
In sugar beet leaves, the follows about 8-10 days aft duction of genes encoding tutive promotor such as 35 against *C. beticola* and oth The genes have been cloned ferent combinations. An enh constructs before the sug *benthamiana* via *Agrobacteri* Chitinase 4, belonging to a in *N. benthamiana*. Sugar b *benthamiana* is therefore electrophoresis.
To examine the antifungal evaluate the degree of resi *ana* have currently been gen will be examined for resist

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